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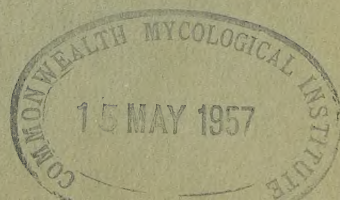
G. C. AINSWORTH and S. D. GARRETT



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SOME NEW MARINE PYRENOAMYCETES ON WOOD OR ROPE: *HALOPHIOBOLUS* AND *LINDRA*

By IRENE M. WILSON

University College of Wales, Aberystwyth

(With Plate 15 and 30 Text-figures)

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*HALOPHIOBOLUS**

Linder (Barghoorn & Linder, 1944) separated the genus *Halophiobolus* from *Ophiobolus* by the marine habitat of all its known species, the predominantly membranous perithecia, the lack of paraphyses and the characteristic conoid, hyaline appendage at each end of the ascospore. The genus was classified according to Saccardo's system in the scolecosporous Sphaeriaceae and distinguished from *Linospora* by the absence of a clypeus and from *Robergea* by the absence of paraphyses.

In Linder's key (Barghoorn & Linder, 1944, p. 416), the main criteria used in the separation of the seven species recognized at that date were

* The generic name *Halophiobolus* is used here in the sense of Linder (1944). Petrak has suggested in *Sydowia* (6, 383-388 1952) that, of the seven species listed by Linder, the short-necked species *H. maritimus* (Sacc.) Linder, *H. medusa* (Ell. & Ev.) Linder and *H. halimus* Mounce & Diehl must be classified in *Linocarpon* Syd., and possibly *H. salinus* Linder also, while the remaining three species with a long projecting neck cannot be separated from *Ophioceras*. He regards the appendages on the spores as of only secondary significance assuming (erroneously in my opinion), that they are merely part of the epispore, ignores the marine habitat of all the described species and is unaware of the similarity in development of both short- and long-necked types with their pseudoparenchymatous centrum, later replaced by asci, and without paraphyses or pseudoparaphyses at any time.

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the length of the perithecial neck in relation to the perithecial venter, the structure and colour of the perithecial wall, the size of the perithecium, the size and septation, if any, of the ascospores together with the size and form of their appendages and the nature of the substrate on which the fungus developed.

The application of these criteria in the identification of the species found here has presented some difficulties owing to the great range of variation within one species. For example, the accommodation of the form of the perithecium to the substrate in which it develops leads to great variation in the size and proportions of the organ. In *Halophiobolus rufus* sp. nov. and *H. purpureus* sp. nov., the venter is frequently elongated along the wood elements parallel to the surface and the neck turns up more or less at right angles to it (Text-figs. 2, 3 and 9). Where the wood is resistant to destruction by the fungus, this elongation can take an extreme form (Text-fig. 2). If such a perithecium is dissected out of the wood it becomes more or less flask-shaped with an almost globose venter and central neck, thus showing that the form in wood was an adaptation to a rigid medium. When the perithecia are produced superficially on wood or on rope, as they may be in *H. purpureus*, the perithecia are usually flask-shaped. The neck also is confined by the wood through which it grows, sometimes taking a sinuous course, and when dissected out, it stretches considerably. When growing superficially the neck may be longer than when the same species is growing immersed in wood. Frequently, owing to erosion, the full length of the neck is not seen. The thickness of the wall also varies according to the substrate. The perithecium is 'woolly' with outgrowing hyphae when it is superficial on wood or rope, less so in the large vessels of angiospermous wood, and relatively smooth and thin in compact gymnospermous wood. The thin layer of crushed wood round the perithecium may easily be mistaken for an outer layer of cells unless it is distinguished by differential staining or by observing from which layer the secondary mycelium arises.

Great variation is also seen in the length of the filiform ascospores. The asci deliquesce and release the spores into the perithecial cavity and considerable growth seems to occur before they are discharged since it is not unusual to find spores which vary in length by 50μ or even 100μ in the same perithecium. The spore measurements given here for new species therefore include stages in which asci as well as loose spores are present. The appendages on the spores, too, may show considerable differences in size according to their state of maturity. Such great variations in spore size seem fantastic in comparison with those of fungi with short spores which are discharged as soon as the asci are ripe.

Linder (Barghoorn & Linder, 1944) does not state whether the spore sizes given for *Halophiobolus* include the appendages or not, but it is assumed here that they do not. If they ought to be included, the two new species described below differ more from Linder's *H. opacus* than is apparent from the spore measurements stated for these species (Table 1). For comparison with Linder's figures, spore measurements should be made in lactophenol, but the diagrams of spores in this paper are of fresh spores in sea water except where otherwise stated.

The unnamed species of *Halophiobolus* referred to by Meyers (1953, 1954) are larger in perithecial and spore size than the species described here.

***Halophiobolus purpureus* sp.nov.** (Text-figs. 1-8, Pl. 15, figs. 2, 3).

Perithecia sparsa vel gregaria, immersa vel superficialia, nigra, sub microscopio autem purpureo-brunnea, $126-339 \times 98-224 \mu$; paries peritheciolorum membranaceus, externe coloratus, interne hyalinus vel subhyalinus, $8-12-(20) \mu$ crassitudine; collum centrale vel non centrale, atro-brunneum vel nigrum, ad apicem pallidum. *Asci* elongato-clavati, octospori, mox deliquescentes. *Paraphyses* nullae. *Ascosporae* filiformes, curvatae vel spiraliter tortae, hyalinae, o-septatae, multivacuolatae, utrimque ad extrema appendicula hyalina conoidea ornatae; ascosporae, appendiculis exclusis, $190-257-(292) \times 3-4 \mu$; appendicululae $10-16-(26) \mu$ longitudine.

Hab. In ligno vel fune in mari submerso.

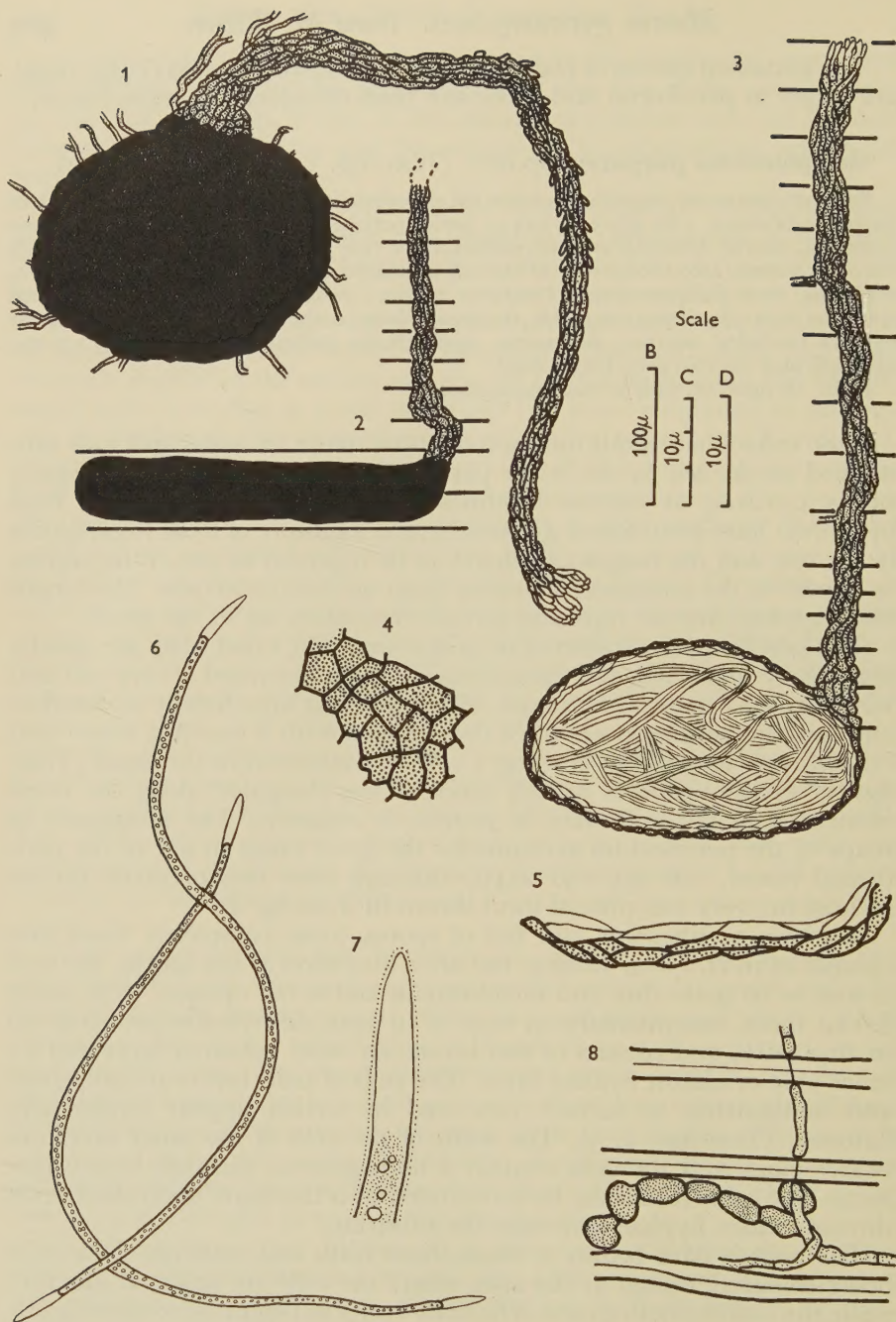
Nine collections of this fungus have been made on wood and rope submerged in the sea in the lower part of the intertidal region or slightly below L.W.M.S., at various localities on the West Wales coast. Ripe perithecia have been found $4\frac{1}{2}$ months after exposure of fresh wood blocks in the sea, and the fungus can therefore be regarded as one of the earliest to appear in the succession of marine fungi on these substrates. The fungus often appears first on rope and spreads from there on to the wood.

The perithecia are scattered or gregarious. On wood, they are usually completely immersed, but exceptionally, when the wood is very soft and wet, they may appear superficial. When growing superficially on wood or on rope, the perithecia are often flask-shaped with a rounded venter and central, very long neck (Text-fig. 1). When immersed in the wood (Text-figs. 2, 3) the venter is usually more or less elongated along the wood elements and the neck may be centric or excentric. The divergence in shape of the perithecium accounts for the great range in size of the perithecial venter, $126-339 \times 98-224 \mu$, although these measurements do not include the very exceptional form shown in Text-fig. 2.

A whole perithecium, still full of spores, looks completely black and opaque as in *H. opacus* Linder, but after liberation of the spores, the wall is seen to be quite thin and membranous and is not opaque. It is about $8-12 \mu$ thick, exceptionally on rope or in open dicotyledonous woods up to 20μ thick, and consists of two layers, an outer coloured layer and an inner pale or almost hyaline layer. The cells of both layers are polygonal and isodiametric in surface view and in section appear tangentially flattened (Text-figs. 4, 5). The walls of the cells of the outer layer are nearly black and the cells contain a homogeneous purplish brown pigment. The outer layer may be surrounded by a thin layer of crushed wood through which hyphae penetrate the substrate.

The neck is dark brown to black, both walls and contents of the cells being coloured, except at the apex where the cells are paler, at any rate while the ostiole is still closed. The neck varies in length up to about 438μ when immersed in wood and may take a somewhat sinuous course, while in a superficial perithecium it may be up to 628μ long. The neck is $15-27 \mu$ wide.

The asci are cylindro-clavate, 8-spored and soon deliquesce. There are no paraphyses among the asci at any stage in development.



Text-figs. 1-8. *Halophiobolus purpureus* sp. nov.

Text-fig. 1. Superficial perithecium on wood. Text-fig. 2. Unusually elongated perithecium in wood, neck incomplete. Text-fig. 3. A normal perithecium in wood, venter cut open to show spores in groups of eight. Text-fig. 4. Surface view of cells of perithecial venter. Text-fig. 5. Section of perithecial wall. Text-fig. 6. Two ascospores. Text-fig. 7. Appendage of ascospore. Text-fig. 8. Mycelium and chlamydospores. Text-figs. 1-3: scale B; Text-figs. 4-6 and 8: scale C; Text-fig. 7: scale D.

The ascospores are filiform, curved or spirally twisted, o-septate, multivacuolate and terminated at each end by a hyaline, conoid appendage (Text-figs. 6, 7). Spores, excluding appendages, measure $190-257 \times 3-4 \mu$ and the appendages are $10-16 \mu$ long. Exceptionally, spores up to 292μ and appendages up to 26μ long have been seen.

The mycelium in the wood is slightly coloured and has a brownish tinge. The hyphae run longitudinally through the cavities of the wood elements and at right angles across them, making bore-holes through the walls (Text-fig. 8). They also penetrate the rays. Infected wood may be darkened and softened near the surface.

In culture on malt and sea-water agar the mycelium forms brown ropy strands with finer, colourless branches and chains of chlamydospores. Chlamydospores are also formed in wood (Text-fig. 8). Experience of the species in the field, so far, suggests that after forming one crop of perithecia it usually survives in the wood as chlamydospores (Text-fig. 8) and is succeeded by other marine species.

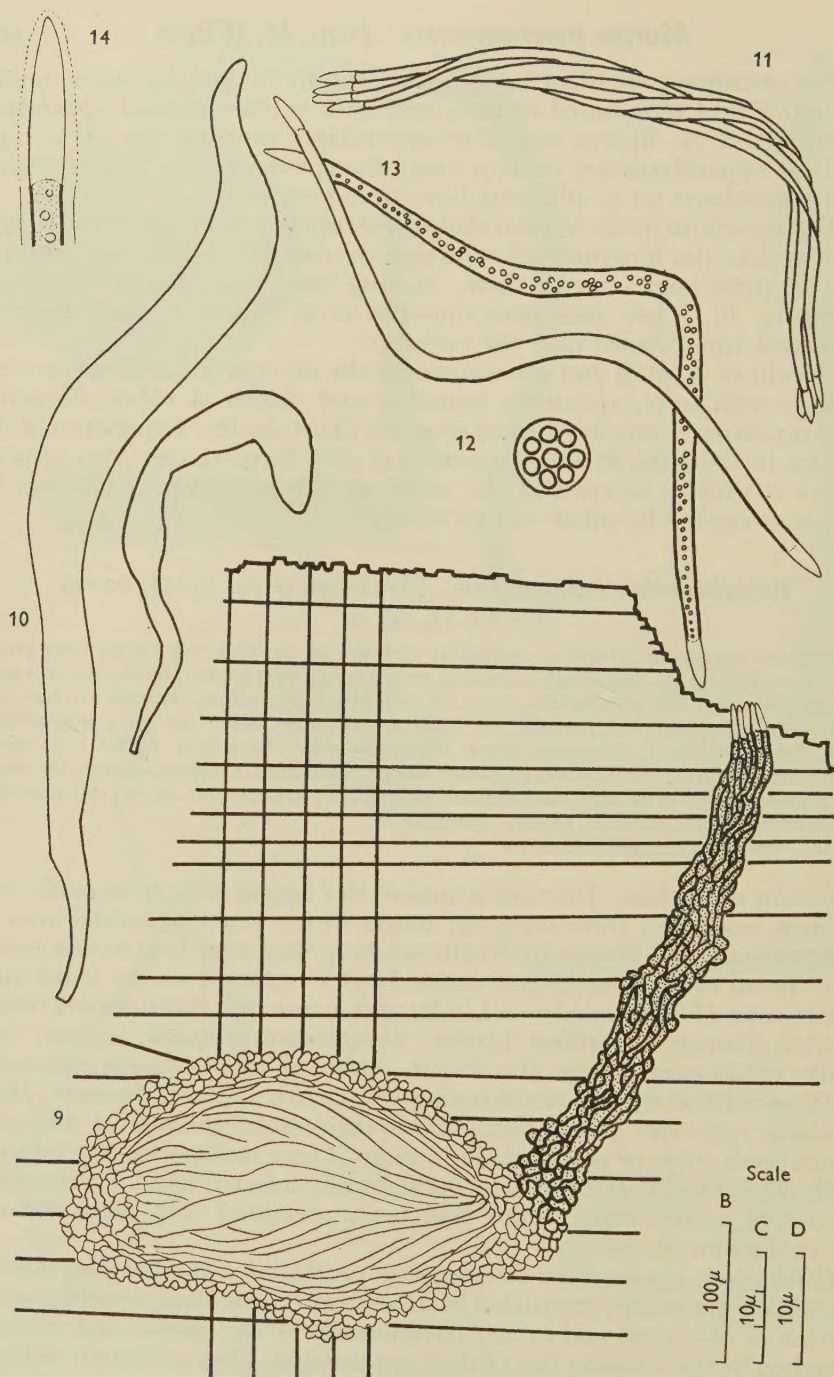
Halophiobolus rufus sp.nov., (Text-figs. 9-14, 15-17, 22-24,
Pl. 15, fig. 4)

Perithecia sparsa vel gregaria, immersa, globosa vel ovoidea secundum ligni strias, $251-355 \times 164-273 \mu$, maturitate subfusca, ante autem rufa; paries peritheciolorum membranaceus, dupliciter compositus, circa 18μ crassitudine; collum centrale vel non centrale, atro-brunneum vel nigrum, ad apicem pallidum, usque ad 426μ longitudine. *Asci* elongato-clavati, octospori, mox deliquescentes. *Paraphyses* nullae. *Ascosporae* filiformes, hyalinae, curvatae vel spiraliter tortae, o-septatae, multivacuolatae, utrimque ad extrema appendicula hyalina conoidea ornatae, ascosporae $216-284 \times 4-6 \mu$ appendiculis exclusis; appendiculae $10-12 \mu$ longitudine.

Hab. In ligno in mari submerso.

Locality and habitat. Four collections of this fungus have been made, one on drift wood and three on wood blocks in the lower intertidal area at Aberystwyth, and always in situations where they were kept continuously wet. In all cases, *Ceriosporopsis halima* Linder occurred on the wood also, in two cases *Helicoma maritimum* Linder and in one case *Peritrichospora integra* Linder, *Remispora maritima* Linder, *Halophiobolus purpureus* sp.nov. and *Lindra inflata* sp.nov. were also found. Although all the blocks eventually became riddled by the wood-boring crustacean *Limnoria lignorum*, *Halophiobolus rufus* and *Ceriosporopsis halima* maintained themselves and produced fresh crops of perithecia for 6 years. These two species also infected fresh wood blocks attached near the naturally infected ones, but the other species of marine fungi which had been associated with them did not infect the new blocks.

Germination, mycelium and development of perithecium. The flexible, filiform ascospores are readily entangled in small filamentous algae growing on the surface of the wood and in any irregularities of the surface and become attached by the adhesive tips of their appendages. They germinate without any resting period, becoming septate, first near the centre and then throughout their length and at the same time elongate considerably. Lateral hyphae may grow out from any of the cells except from the terminal appendages (Text-fig. 15).



Text-figs. 9-14. *Halophiobolus rufus* sp. nov.

Text-fig. 9. Perithecium in wood. Venter cut open to show asci. Text-fig. 10. Mature and immature asci in outline. Text-fig. 11. Group of eight ascospores. Text-fig. 12. T.S. ascus showing arrangement of spores. Text-fig. 13. Two ascospores. Text-fig. 14. Appendage of ascospore. Text-fig. 9: scale B; Text-figs. 10-13: scale C; Text-fig. 14: scale D.

The mycelium found in the wood (Text-fig. 16) is very characteristic. The main strands are grey with a pinkish tinge. In coniferous wood they run for considerable distances along the cavities of the tracheids. Many lateral strands take an oblique course across the tracheids forming narrow bore-holes through the walls or utilizing the bordered pits. The rays are also penetrated, hyphae travelling along the tracheidal cells and ramifying within the parenchymatous cells of the ray. Chains of chlamydospores may be formed.

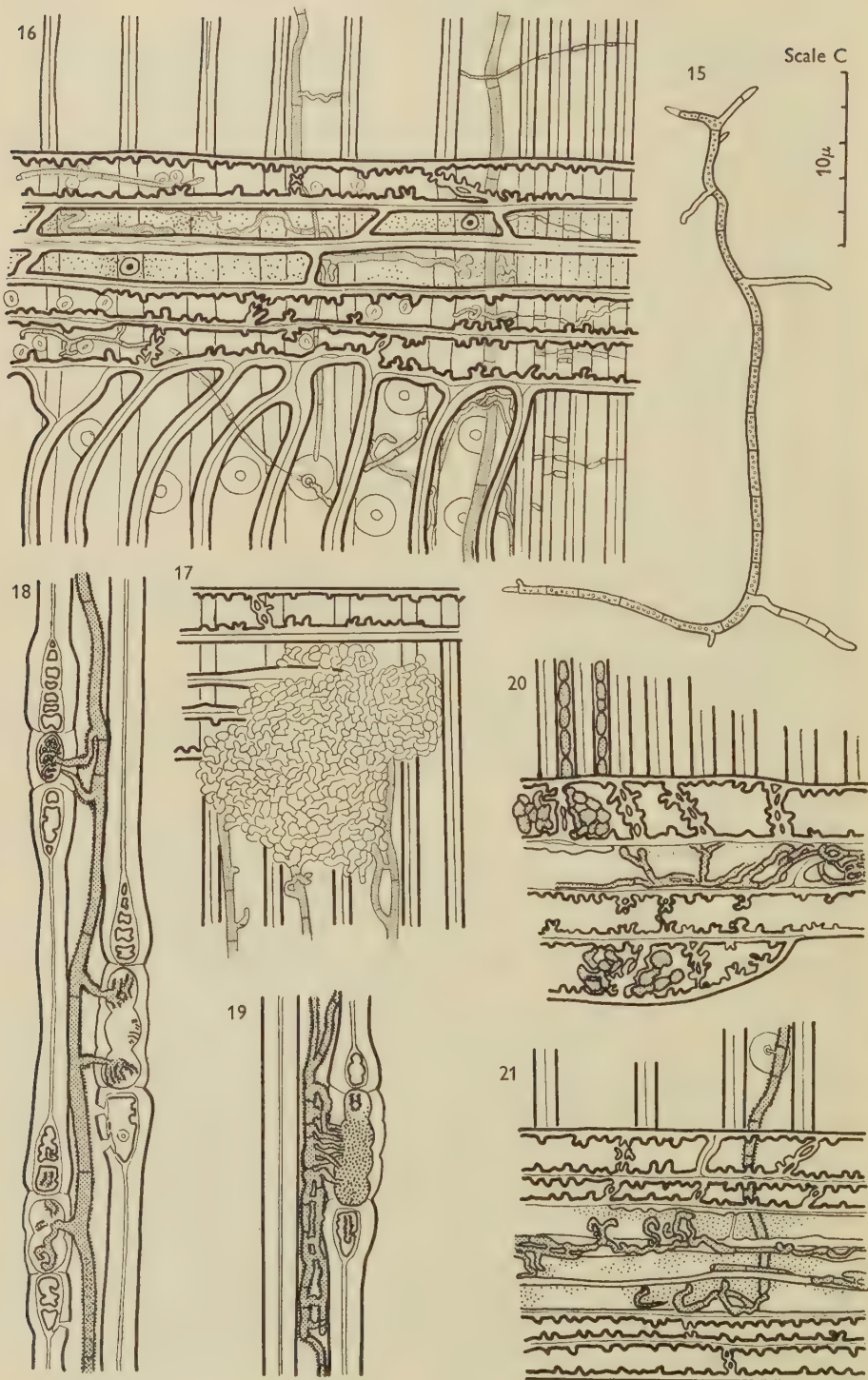
After staining naturally infected with Bismarck Brown and Gentian Violet or with Safranin and Picro-aniline Blue it can be seen that hyphae also run within the wall itself, especially of the thick-walled summer tracheids (Text-fig. 22). Using the terminology for the parts of the wall adopted by Kerr & Bailey (1934), these hyphae are confined to the middle part of the secondary wall which is characterized by containing a considerable amount of cellulose and is less lignified than the rest of the wall. They turn in steep spirals and apparently follow the cellulose macro-fibrils (Text-fig. 23). *H. rufus* is thus probably similar to *H. longirostris* and *Ceriosporopsis halima* studied by Barghoorn (Barghoorn & Linder, 1944), and to many other wood-destroying fungi.

The earliest stage seen in the development of the perithecium consists of an irregular ball of pseudo-parenchymatous tissue of a bright pink colour, often situated at an angle between a ray and the xylem elements. Continuity with the pinkish grey mycelium can be traced and there are frequent hyphal fusions in the vicinity (Text-fig. 17).

The development of the perithecium has not been followed in detail but after the main form of the perithecium has been established and before the asci appear, it has been observed that the centrum is filled with a pseudo-parenchymatous tissue of large, rounded cells about $12-16\mu$ in diameter.

The young perithecium is characterized by the possession of a bright orange-red pigment (hence the specific name) which colours the neck, perithecium wall, asci and ascospores. The first part to lose this pigmentation is the neck which soon becomes a dark brown colour, both walls and contents of the outer layer of cells being darkly coloured. The tip of the neck remains paler than the rest and appears to be closed until just before spore discharge.

The mature perithecium. The form usually taken by the mature perithecium which is completely immersed in the wood might be described as swan-shaped and measures $306-355 \times 164-202\mu$ (Text-fig. 9), but it is sometimes more flask-shaped and it is then $254-273 \times 219-273\mu$. The wall of the venter is of two layers and is about 18μ thick. The outer layer consists of two to three layers of small isodiametric cells with thick, shining walls which darken somewhat at maturity giving the perithecial venter a dingy colour after the red pigment, which marked the earlier stage, has disappeared. The inner layer is of thin-walled, hyaline cells flattened tangentially. This inner layer stains with Congo red while the outer layer does not. Spore discharge occurs when the perithecial venter is pale fuscous and the neck dark brown and this is therefore believed to be the mature condition.



Text-figs. 15-21

The neck is up to 426μ long and is much darker than the venter, with paler hyphae at the apex.

The asci are cylindro-clavate, often curved and rather pointed at the top (Text-fig. 10). The wall is thin and there is no indication of a bitunicate structure in the sense of two separable membranes. The apex is difficult to observe on account of the dense red pigment in the immature ascus and the early deliquescence, but no pore was observed although there was some indication of a shallow ring-like thickening on the inner wall and a highly refractive spot near the apex. The asci are 8-spored and soon deliquesce.

The ascospores are filiform, aseptate, curved or twisted and multi-guttulate, with a terminal, hyaline, conoid appendage at each end (Text-figs. 11-14). The spores measure $216-284 \times 4-6\mu$ (excluding appendages), and the appendages are $10-12\mu$ long. The whole spore, including the appendages, is covered by a shining refractive layer. At the time of spore discharge it is only the apex of the appendage which is sticky.

Spore discharge. Spore discharge was observed in a perithecium partly dissected out of the wood and immersed in sea water on a slide without a cover-slip (Text-fig. 24). The spores were discharged one at a time, at first in short runs of eight, and then continuously for over an hour. The rate was about twenty spores in a period of 3 min. One end of the spore slips slowly out of the ostiole until it is about half out, then it bends down and round in swift and violent twisting movements in a plane at right angles to the neck, then up again in line with the neck, bent or corkscrewed, and glides away from the ostiole. While straightening and gliding away, the next spore has already started to slip out. Text-fig. 24 represents diagrammatically the successive positions of three spores drawn with a camera lucida during discharge. In one section of a perithecium a spore was seen doubled in half in the neck but it is not thought that this normally occurs since two spores have sometimes been seen to come out together, stuck together apically by their appendages. The exact mechanism of the action needs further study, especially the cause of the remarkable contortions of the very flexible spores.

Halophiobolus longirostris Linder (Pl. 15, fig. 5)

This species, not previously recorded for the British Isles, was found on 7 August 1953 on the remaining stump of a wooden stake driven into the sand near L.W.M. at Tyrella, Co. Down, Northern Ireland. Probably this stake was the remains of the wartime defence system and had been there since about 1940. It was very rotten and was silted up with sand and there was some algal growth on the surface also. Beneath this crust were the

Legends to Figs. 15-21

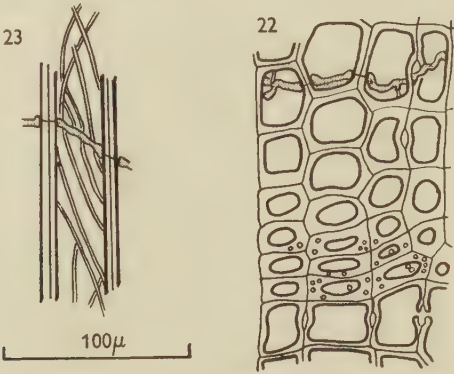
Text-figs. 15-17. *Halophiobolus rufus* sp. nov.

Text-fig. 15. Germinating spore. Text-fig. 16. Mycelium in R.L.S. secondary xylem and ray of *Pinus* sp. Text-fig. 17. Young perithecium.

Text-figs. 18-21. *Lindra inflata* sp. nov.

Text-figs. 18, 19. Mycelium with haustoria into rays in T.L.S. wood of *Pinus* sp. Text-fig. 21. Mycelium destroying cell walls of parenchymatous cells of ray in R.L.S. *Pinus* wood. Text-fig. 20. Chlamydospores in summer tracheids and ray tracheids of *Pinus* sp. R.L.S. All figs.: scale C.

perithecia of *H. longirostris* immersed in the wood and large, brownish, rounded cells (chlamydospores?) were also present, similar to those in the type material. There were also smaller chlamydospores present like those of *H. purpureus* indicating that the wood had been infected by another species previously.



Text-figs. 22, 23. *Halophiobolus rufus* sp. nov.

Text-fig. 22. T.S. wood of *Pinus* sp. through annual ring showing coloured hyphae traversing tracheids, making bore-holes through the walls or utilising the bordered pits, and fine colourless hyphae, cut transversely, in the middle region of the secondary wall of the late-formed summer tracheids. Text-fig. 23. T.L.S. *Pinus* wood showing coloured hyphae as in Text-fig. 22 and the colourless hyphae following a steep spiral within the middle layer of the secondary wall.

H. longirostris has an extremely long, black neck measuring up to 700 μ and a rather paler venter measuring, in this material, 363–477 $\times$ 185–369 μ . There were no asci present, but the ascospores and their appendages agreed in size with those of the type material.

Table 1. Comparison of *Halophiobolus opacus* Linder, *H. purpureus* sp. nov., *H. rufus* sp. nov. and *H. longirostris* Linder. All measurements in μ

Species	Colour	Perithecium			Spores	
		Venter	Neck	Wall	Spore	Appendage
<i>H. opacus</i> Linder*	Black and opaque	198–264 $\times$ 148–214	300 $\times$ 28–33	11.5–16.5– (21.5)	155–190 $\times$ 3.3–4	7.5–8.5
<i>H. purpureus</i> sp. nov.	Purplish brown to black	126–339 $\times$ 98–224	628 $\times$ 15–27	8–12–(20)	190–257– (292) $\times$ 3–4	10–16– (26)
<i>H. rufus</i> sp. nov.	Neck dark, venter hyaline to sub-fuscous	251–355 $\times$ 164–273	426 $\times$ 27–33	c. 18	216–284 $\times$ 4–6	10–12
<i>H. longirostris</i> Linder*	Neck dark, venter subhyaline or dilute fuscous	362–477 $\times$ 185–369	594–700 $\times$ 41–58	—	155–200 $\times$ 3.3–4	7–8

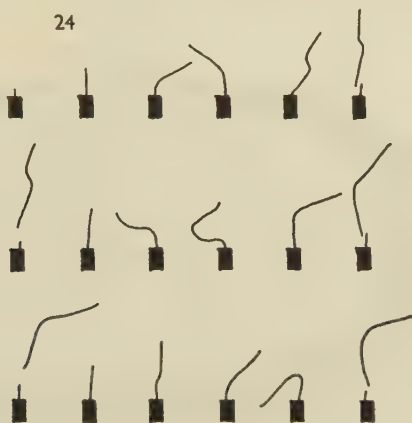
* From Barghoorn & Linder (1944) except venter size in *H. longirostris*.

Comparison

H. purpureus differs from *H. opacus* in the slightly larger perithecia with much longer and thinner necks, the thinner, less opaque and more purplish wall and the longer spores with longer appendages. Examination of

the type material of *H. opacus* (Pl. 15, fig. 1) fully confirms Linder's description (Barghoorn & Linder, 1944) and there is no indication in this material that the spores and appendages ever reach the dimensions of those of *H. purpureus*. If, however, further collections of older material of *H. opacus* should reveal that such a variation exists, this new species should be abolished or reduced to a variety. Meanwhile it is necessary to give it a name.

H. rufus differs from *H. purpureus* in the more robust perithecium, the shorter, thicker neck and the paler colour of the perithecial venter. The spores are about the same length as those of *H. purpureus* but are wider. The pale venter resembles that of *H. longirostris* but there are no other similarities between the two species.



Text-fig. 24. *Halophiobolus rufus* sp. nov. Spore discharge. Camera lucida drawings of three spores during spore discharge.

LINDRA

Lindra gen. nov. *Perithecia* solitaria vel gregaria, aliquando coniuncta, semiglobosa vel late rotundata, paries peritheciorum niger et crassus, collum brevissimum vel nullum; *asci* cylindro-clavati, octospori, mox deliquescentes; *paraphyses* nullae; *ascosporae* filiformes, hyalinae, septatae et multivacuolatae, utrimque ad extrema appendicula hyalina, globosa vel semi-globosa, ornatae.

Ab *Halophiobolo* pariete perithecii et appendiculis sporae differt.

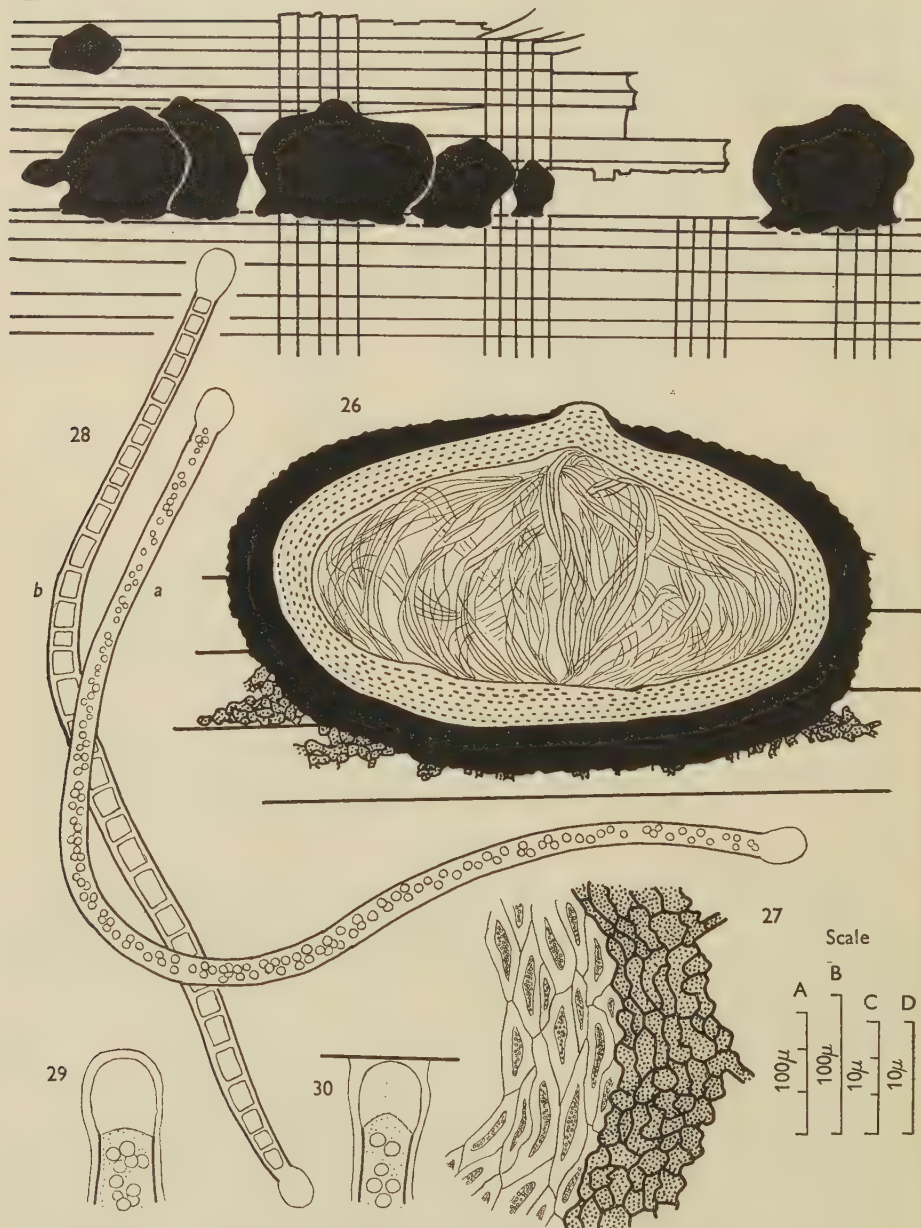
Typus:

Lindra inflata sp. nov. (Text-figs. 18-21, 25-30, Pl. 15, fig. 6)

Perithecia solitaria vel in series breves disposita, aliquando coniuncta, primum immersa, deinde substrato attrito vel paulatim confracto superficialia, semi-globosa vel late rotundata, externe nigra et opaca, $339-535 \times 250-375 \mu$; paries peritheciorum $40-60 \mu$ crassitudine, externe niger, interne hyalinus; ostiolum papilliforme, centrale vel non centrale. *Asci* cylindro-clavati, octospori, mox deliquescentes. *Paraphyses* nullae. *Ascosporae* filiformes, hyalinae, multivacuolatae, $30-50$ -septatae, $212-411 \times 4-6 \mu$ ($290 \times 5 \mu$) appendiculis exclusis; appendiculae hyalinae et globosae vel semi-globosae, $8-12 \times 6-10 \mu$.

Hab. In ligno in mari submerso.

The type material of *Lindra inflata* is taken from a wood block attached near the lower part of the intertidal region at Aberystwyth. It occurred

Text-figs. 25-30. *Lindra inflata* sp. nov.

Text-fig. 25. R.L.S. of wood showing perithecia. Text-fig. 26. Diagrammatic L.S. of one perithecial wall showing ascospores in groups of eight. Text-fig. 27. Portion of perithecial wall in section. Text-fig. 28a. Ascospore mounted in sea water. Text-fig. 28b. Ascospore mounted in glycerine jelly. Text-fig. 29. Appendage of ascospore. Text-fig. 30. Appendage of ascospore. Text-fig. 25: scale A; Text-fig. 26: scale B; Text-figs. 27, 28: scale C; Text-figs. 29, 30: scale D.

on the under part of the block where the wood was very damp, and it was associated in part with *Halophiobolus purpureus* and *H. rufus*, following these species in the succession of forms attacking the wood. Other collections have been made from driftwood and from the wooden piles under the jetty at Aberystwyth.

The mycelium is very conspicuous even before the perithecia appear. The main hyphae are dark brown, with a rough surface (Text-figs. 18–21). In Pine wood they run for considerable lengths through the xylem tracheids sending numerous, hyaline, haustorium-like branches into the central, parenchymatous cells of each of the rays. After attacking these the hyphae, now brown, pass into the tracheidal cells of the ray and hence to fresh areas. Eventually the rays may be completely destroyed, both walls and contents of the cells being replaced by the dark brown, branching and anastomosing hyphae of the fungus. The mycelium has also been seen in the resin canals. Chains or irregular masses of dark brown chlamydospores (Text-fig. 21) are formed in the cavities of the xylem elements, in resin canals and the rays, especially in the tracheidal cells, of coniferous wood. Attacked wood is soft and blackish in colour.

The perithecia are formed completely immersed in the wood, singly, or more often, contiguously in short series and sometimes joined to each other laterally. They become exposed by the erosion, or the splintering off, of the overlying wood (Text-fig. 25). When only the saucer-shaped bases of the perithecia remain, they look superficially like the apothecia of a small black Discomycete.

The perithecia are semi-globose or broadly rounded in shape and flattened at the base where the strands of the brown mycelium, described above, can be seen (Text-figs. 25, 26). There are sometimes curious lateral outgrowths present. The perithecia are black and opaque, and somewhat hard externally when dry. The ostiole is centric or excentric, and, if raised, papillate. It probably opens by disintegration of the cells.

The black perithecia vary in size from $339\text{--}535 \times 250\text{--}375 \mu$. The smallest of these may be immature. Before the formation of asci, the centrum is filled with a hyaline pseudo-parenchymatous tissue of thin-walled, polygonal, isodiametric cells containing minute oil drops and a 'squash' at this stage also reveals a few very much larger globose cells with dense oily contents which, however, soon swell and gelatinize when mounted fresh in sea water.

The perithecial wall is extremely thick, $40\text{--}60 \mu$, and consists of two layers (Text-figs. 26, 27). The outer part appears black in thick sections and consists of several layers of cells with very dark walls and contents. From the outer cells of this layer the characteristic brown hyphae can be seen. The inner layer of the wall consists of hyaline cells, rhomboidal in section, with thick, colourless walls and containing numerous small yellowish oil drops.

The asci are cylindro-clavate, thin-walled and 8-spored. No apical pore or thickening has been seen. The asci soon deliquesce releasing the spores into the perithecial cavity and are therefore rarely seen.

No paraphyses are formed at any stage of development. The pseudo-parenchymatous tissue of the centrum disappears as the asci develop.

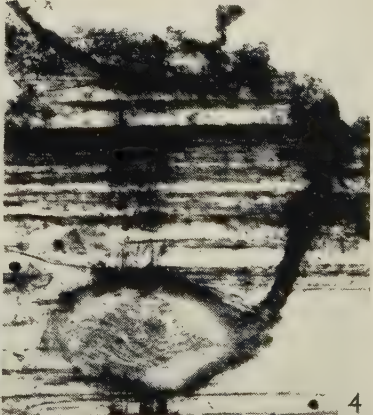
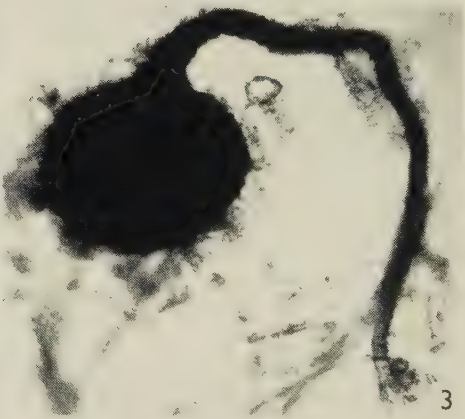
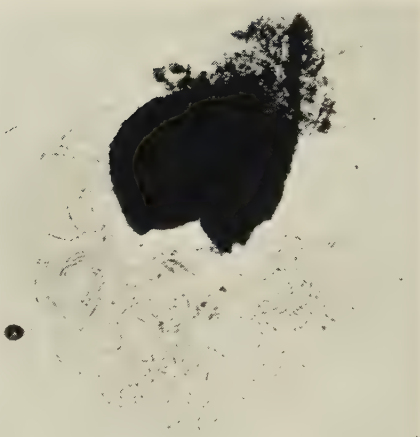
The ascospores are filiform, hyaline, $212-411 \times 4-6 \mu$ (average $290 \times 5 \mu$), multivacuolate and inconspicuously and rather irregularly septate (Text-fig. 28). The septa may not be detected if spores are mounted fresh in sea water (Text-fig. 28*a*). The septa can readily be seen, however, by transferring to glycerine jelly (Text-fig. 28*b*), or by staining with Congo red or with acid Fuchsin lactophenol. There are thirty to fifty transverse septa in each spore. At each end of the spore is a globose or semi-globose, balloon-like, hyaline appendage, gelatinous at the tip (Text-figs. 28-30). The whole spore has a shining outer rim. The spores at first sight look very much like the hormogones of a blue-green alga with their shining, greenish, oily contents and their almost uniform width.

COMPARISON OF *LINDRA* AND *HALOPHIOBOLUS*

The proposed new genus *Lindra* seems to be closely related to *Halophiobolus* in structure and development. The principal difference lies in the spores which have conoid appendages in *Halophiobolus* and globose or semi-globose appendages in *Lindra*. The spores of *Halophiobolus* are serpent-like, tapering gradually to each end, while in *Lindra* they are of the same width for the greater part of their length. The spores are septate in *Lindra* but are aseptate in all species of *Halophiobolus* except *H. maritimus* (Sacc.) Linder where they are 15- to 18-septate and break up into uniseptate segments. This latter species was first described by Saccardo (1878) as *Rhaphidophora maritima*, and it was stated that it occurred on material collected by P. Magnus on *Zostera marina*. According to Tutin (1934), however, it occurs, 'unter' not 'auf', *Zostera* on the leaf of a grass which is not *Zostera* but probably *Elymus*. *Rhaphidophora maritima* Sacc. was then transferred to *Ophiobolus* (Saccardo, 1883) becoming *Ophiobolus maritimus* (Sacc.) Sacc.* Linder (Barghoorn & Linder, 1944) transferred it to *Halophiobolus*, without stating any reasons, but presumably because of its marine habitat. It seems doubtful whether it should really be included in *Halophiobolus* because the septate, fragmenting spores are not found in any other species, and it also appears to lack the hyaline conoid appendages (Saccardo 1878, 1883) which are a diagnostic feature of the genus *Halophiobolus* Linder. If *H. maritimus* (Sacc.) Linder were excluded from *Halophiobolus*, there would be a clear distinction between the genus *Halophiobolus* with filiform, hyaline, aseptate, serpent-like spores with conoid appendages and *Lindra* with filiform, hyaline, septate spores of uniform width with globose or semi-globose appendages.

The perithecium wall is the second major point of difference between *Halophiobolus* and *Lindra*. In the former genus the wall is typically membranous and subhyaline to black, while in *Lindra* it is extremely thick, and black and may become rather hard when dry. Moreover, the perithecia of *Lindra* seem to be occasionally joined, two or three together, by a common wall, a phenomenon not recorded for *Halophiobolus*. Alter-

* Berlese (1899) borrowed the type from Saccardo's herbarium but could not find the species on it. Butler re-examined the specimen for Tutin, who refers, only to the host, not the fungus (Tutin, 1934). The Director of the Museum at Berlin-Dahlem informs me that the type specimen was destroyed in January 1943.



native interpretations of this might be: (1) perithecia with more than one ostiole, as in 'Form 2' of the marine fungi described by Meyers (1953); or (2) the centra as locules in a stroma. Only a more detailed study of development can decide between these different possibilities.

For the rest, the two genera have much in common, with a pseudo-parenchymatous centrum at an early stage, replaced later by 8-spored asci which soon deliquesce and liberate filiform ascospores with an appendage at each end. Both genera form chlamydospores. Both are marine and contain one or more species attacking wood.

I am grateful to Prof. W. H. Davies for the Latin diagnoses, to the Director of the Farlow Herbarium and Library, Cambridge, Massachusetts, for the loan of type specimens and to Mr E. W. Mason for drawing my attention to the reference from Berlese. Type material has been deposited in the Commonwealth Mycological Institute, Kew, Surrey, by kind permission of the Director.

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EXPLANATION OF PLATE 15

(All about the same magnification.)

- Fig. 1. Perithecium of *Halophiobolus opacus* Linder.
 Fig. 2. *H. purpureus* sp.nov. Perithecium burst to show ascospores.
 Fig. 3. Perithecium of *H. purpureus*.
 Fig. 4. Perithecium of *H. rufus* sp.nov. in wood.
 Fig. 5. Perithecium of *H. longirostris* in very rotten wood. Neck incomplete.
 Fig. 6. *Lindra inflata*. Two joined perithecia, one cut open showing spores.

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LICHENOLOGICAL NOTES. I.

BY A. E. WADE

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Many new county records are given. *Physcia dubia* (Hoffm.) Lettau is recorded for the first time as occurring in Britain. The name *Lecanora conizaea* Nyl. is shown to have been wrongly applied in Britain. Some nomenclatural changes of the names in the *Census Catalogue* are made and the following new combinations proposed: *Hypogymnia tubulosa* (Schaer.) and *Caloplaca aurantia* (Pers.) Hellb. var. *brevilobata* (Nyl.).

The county records given in the following notes are additional to those in Watson's *Census Catalogue of British Lichens*, 1953, and are based upon specimens sent to me by correspondents, personal records, or upon specimens in the Welsh National Herbarium, National Museum of Wales. The latter are indicated by an exclamation mark (!). I am responsible for records given without an authority. Some nomenclatural changes are referred to and one species, *Physcia dubia*, is here recognized for the first time as occurring in Britain. Notes are also included which it is hoped may be helpful in the discrimination of some closely related species.

Parmelia fuliginosa Nyl. 52, Rhoscolyn, 1954, P. W. James.

Parmelia borreri (Turn.) Turn. Berry (1941, 90) has pointed out that the citation of *P. dubia* by Schaerer as a synonym of *P. borreri* was based on a typographical error, and that *Lichen dubius* Wulf apud Jacq. (1790) has powdery soredia on the margins and is not the plant described as *P. borreri* by Turner. The latter name must, therefore, replace *P. dubia* Schaer.

Hypogymnia tubulosa (Schaer.) comb.nov. (*Parmelia ceratophylla* var. *tubulosa* Schaer. *Enum.* **42**, 1850). British lichenologists have in the past treated this as a variety of *Hypogymnia physodes* (L.) Wats. (*Parmelia physodes* (L.) Ach.). It is, however, worthy of the specific rank given to it by continental lichenologists since it differs from *H. physodes* in the form of the thallus, in the terminal, capitate soralia, and in the negative reaction of the medulla and soralia to Pd. In *H. physodes* the chemical reaction is Pd⁺ yellow turning orange.

4, on apple, Berrynarbor 1908, C. E. Larter. ! 41, Pen-y-lan, Cardiff, 1932. ! 44, on Scots pine, near Blaen Cib., Llandilo, 1954. ! 51, on granitic rock, Hope Mountain, 1955, P. W. James. ! 52, Rhoscolyn, J. E. Griffith. !

Platysma chlorophyllum (Humb.) Wats. 64, on ash, etc. Malham Tarn, 1955.

Lecanora galactina Ach. var. *dispersa* (Pers.) Ach. 48, Lake Bala area, 1953, P. W. James.

Lecanora chlarona (Ach.) Cromb. 28, on *Salix atrocinerea*, Swangey Fen, near Shropham. ! This species may be distinguished from the others of the *L. subfusca* group by the red reaction of Pd on the thalline margin of the apothecium.

Lecanora subrugosa Nyl. 88, Glen Lochay, 1913, J. A. Wheldon. 98, on alder, shore of Loch Awe near North Port, Sonachen, 1940, I. M. Lamb (1942, 321). This species was overlooked by Watson (1953). It is closely related to *L. subfusca* (L.) Ach., but the apothecia are thicker and more urn-like, with crenulate or strongly granular thalline margins, and the thallus is thicker and more coarsely granulated. Like *L. rugosella* A. Zahlbr., the medulla contains numerous large crystals, but it may be distinguished from that species by the dark reddish brown apothecia and by the absence of a granular epithecium. Chemical reactions: K^{2+} yellow, Pd^{2-} .

Lecanora rugosella A. Zahlbr. (*L. rugosa* auct. plur. non *Lichen rugosus* Pers.). 46, on oak, Allt-yr-Hwch, Dalar-wen, 1952. !

Lecanora crassula H. Magn. 98, on alder, shore of Loch Awe near North Port Sonachan, 1940, I. M. Lamb (1942, 319). This addition to the British Lichen flora was overlooked by Watson (1953). As pointed out by Lamb, it is recorded for Ireland by Magnusson, but without exact locality. The following specimens in the Welsh National Herbarium appear to come under this species. 5, on ash, Asholt, 1914, W. Watson. 35, on beech, between Bedwas and Mynydd-y-grûg, 1924. 42, on hazel, Llan-wrtyd Wells, 1929. 67, East Allendale, 1878, W. Johnston. 69, Naddle Forest, 1903, A. Wilson and J. A. Wheldon. 70, Ashby and Ennerdale, W. Johnson *exsicc.* No. 260 as *L. chlarona* var. *geographica* Nyl. 71, Glen Roye, 1920, J. A. Wheldon. The granular epithecium places *L. crassula* in the *L. rugosella* group, but it differs from the other species in that group in the rather less granular thallus and in the whitish, entire or slightly crenulate, thalline margin of the apothecium. The apothecia are less crowded than is usual in either *L. rugosella* or *L. chlarotera*.

Lecanora chlarotera Nyl. 1, on beech, near Poltesco, 1927, H. H. Knight. ! 46, on oak, Allt-yr-Hwch, Dalar Wen, 1952. ! 67, Chollerford, W. Johnson, *exsicc.* No. 37, as *L. chlarona*. (This removes the brackets from the *Census Catalogue*.) 88, near Loch Low, Dunkeld, 1921, J. A. Wheldon and A. Wilson. ! 96, on chestnut, Tomartin, 1942, U. Duncan. This species has been much confused with others of the *L. subfusca* group. It may be distinguished from *L. allophana*, *L. subfusca* and *L. chlarona* by the granular epithecium, and further from the latter by its negative reaction to Pd. The apothecia, which are often crowded, vary in colour from pale-reddish to yellowish brown. The thallus is rather thick, granular, whitish, yellowish or greyish and may become sorediose in places. It is closely related to *L. rugosella*, which, however, has a less granular thallus and rather thicker apothecia with more strongly rugose thalline margins. Intermediates seem to occur between *L. chlarotera* and *L. rugosella*, and for this reason Poelt's (1952, 67-68) reduction of the latter and *L. crassula* to forms of *L. chlarotera* may be justified.

Lecanora intumescens (Rebent.) Koerb. 42, on ash, Allt Cynhelyn, Llanddewi'r Cwm, 1927. ! On oak, Abergwesyn, 1929. ! Forms of *L. subfusca* and related species with rather thick, more or less entire, apothecia margins have been frequently mistaken for *L. intumescens*, but the latter may be readily separated from such by the deep yellow or orange reaction of Pd on the margin of the apothecium.

Lecanora varia (Ehrh.) Ach. This species is apparently rare in Britain

and most records refer to *L. conizaeoides* Cromb. The chemical reaction of the thalline margin of the apothecium is Pd^+ yellow.

Lecanora conizaea Nyl. This species does not appear to occur in Great Britain or Ireland, but forms of *L. conizaeoides* Cromb. with the thalline margin of the apothecium pulverulent have been confused with it. Acharius (1810, 374) described *L. expallens* var. *conizaea* and gave as localities 'in cortice Pini ae ligno Quercus vetusto Angliae, Turner; Helvetiae, Schleicher'. In addition to the specimen sent by Dawson Turner to Acharius, there is in the latter's herbarium at Helsinki one from the Rev. John Harriman labelled 'Anglia'. Both of these specimens are clearly related to, and scarcely distinguishable from, *L. expallens* as now understood, and give a deep yellow reaction with CaCl . Unfortunately Turner's specimen is sterile. An examination of Nylander's specimens and other material in the Helsinki Herbarium and of specimens in the British Museum Herbarium shows that *L. conizaea* of Nylander and other continental lichenologists is not the same as *L. expallens* var. *conizaea* Ach. from which it differs, amongst other characters, in the negative reaction to CaCl . *L. conizaeoides* Cromb. is readily separated from *L. conizaea* Nyl. and *L. expallens* Ach. by the chemical reaction Pd^+ brick red. In the two latter species there is no reaction.

The lichen described by S. F. Gray (1821, 1, 454) as *Rinodina conizaea* seems, as far as one can judge by the short description, to be the same as *Lecanora conizaeoides* Cromb. Gray's specific epithet, however, cannot be restored, since it would create a later homonym of *L. conizaea* Nyl. Specimens of *L. conizaea* Nyl. collected by Larbalestier in Jersey are correctly named.

Lecanora farinaria Borrer, *Engl. Bot.* (Suppl.) t. 2727, 1832. This was treated by A. L. Smith (1918, 291) as synonymous with *L. conizaea* of British authors, but an examination of a specimen of Borrer's in the British Museum Herbarium shows that it differs from that species in its thick, whitish, farinose thallus, and in the chemical reaction Pd^+ yellow. In view of this it is better maintained as a distinct species. In Sowerby's *English Botany* *L. farinaria* was said to be not uncommon on old rails in Sussex, but it now seems to be very rare, if not extinct. Crombie (1879) referred to it as 'very common on old pales in the north of Middlesex and south of Hertfordshire, though often sterile', but he may have confused it with *L. conizaeoides*.

Lecanora conizaeoides Cromb. 48, Bala Lake area, 1954, P. W. James.

The name *L. pityrea* Erichs. has been widely adopted on the Continent, but as was pointed out by Watson (1933) specimens named *L. pityrea* by Erichsen appeared to be identical with *L. conizaeoides* Cromb. A comparison by the writer of Crombie's specimens in Nylander's herbarium with those named *L. pityrea* by Erichsen confirms their identity.

Psora lurida (Swartz) DC. 41, Old Castle Down, 1955. R. B. I. Cook and A. E. Wade.

Psora ostreata Hoffm. 41, on oak, near Lisvane, 1953, A. E. Wade. !

Lecidea subsequens Nyl. 41, on calcareous stones, Sully Island, 1955. !

Lecidea soredizodes (Nyl.) Wain. 41, old quarry, Clyne Common, 1944, A. E. Wade. !

- Pertusaria lactea* (L.) Nyl. 43, Aberedw Rocks, 1927. !
Pertusaria multipuncta (Turn.) Nyl. 42, on hazel, Abernant, Llanwrtyd, 1923, H. H. Knight. !
Pertusaria leioplaca (Ach.) Schaer. 46, near Dalar-wen, Dothie-Camddwr, 1952. !
Pertusaria sulphurea (Tayl.) Schaer. 42, on sandstone, Craig Cerig-leisiad, 1953. !
Pertusaria henrici (Harm.) Erichs. 41, on sycamore, near Old Castle Down, Ogmere, 1955. !
Pertusaria hemisphaerica (Floerke) Erichs. 48, base of oak Glanllyn Estate, Bala, 1954, P. W. James. 88, Finlarig, J. A. Wheldon and A. Wilson. !
Ramalina pollinaria (West.) Ach. 48, Bala Lake area, 1954, P. W. James.
Ramalina evernioides Nyl. 48, Bala Lake area, 1954, P. W. James.
Thalloidima coeruleonigricans (L.) Th. Fr. 48, Bala Lake area, 1954, P. W. James.
Biatorina cyrtella (Ach.) Th. Fr. 48, on sycamore, Glan Llyn, 1954, P. W. James.
Bacidia inundata (Fr.) Koerb. 64, on sandstone boulder in Darnbrook Beck, Fountains Fell, 1955, A. E. Wade. !
Lecanactis premnea (Ach.) Wedd. 42, Llanbedr, 1954. !
Xanthoria polycarpa (Ehrh.) Oliv. 48, Bala Lake area, 1954, P. W. James.

Placodium DC. This name being a later homonym is illegitimate and must be rejected. The valid name is *Caloplaca* Th. Fr., which in Watson's *Census Catalogue* was restricted to the non-lobulate species. The names under *Placodium* in the above Catalogue should be emended as follows:

- Caloplaca murorum* (Hoffm.) Th. Fr.
C. pusilla (Massal.) A. Zahlbr. (*Placodium murorum* (Hoffm.) DC. var. *pusillum* (Massal.) Flag.).
C. decipiens (Arn.) Jatta.
C. dissidens (Nyl.) Mér.
C. elegans (Link) Th. Fr.
C. fulgens (Swartz) Koerb.
C. aurantia (Pers.) Hellb. var. *aurantia* (*C. callopisma* (Ach.) Th. Fr.).
var. *heppiana* (Müll. Arg) Poelt (*Placodium callopismum* (Ach.) Mér. var. *plicatum* (Wedd.) Leight.).
var. ***brevilobata*** (Nyl.) comb.nov. (*Lecanora brevilobata* Nyl. in *Flora*, 66, 99, 1883).
C. scopularis (Nyl.) Lett.
C. cirrochroa (Ach.) Th. Fr.
C. granulosa (Müll. Arg) Jatta.
C. miniatula (Nyl.) A. Zahlbr.
C. marina Wedd. (*C. lobulata* auct. non *Lecanora lobulata* Floerke).
C. microthallina Wedd.
C. erythrocarpa (Pers.) Th. Fr.
C. xantholyta (Nyl.) Jatta.
C. medians (Nyl.) Flagey.

The citation of Floerke in the *Census Catalogue* as the authority for the specific epithet in the combination *Placodium lobulatum* is erroneous. *Lecanora lobulata* Floerke (1815) is synonymous with *Xanthoria lobulata* (Floerke) Bouly de Lesdain and antedates *L. lobulata* of Sommerfeld (1826), who should have been cited.

Caloplaca pusilla (Massal.) A. Zahlbr. (*Placodium murorum* (Hoffm.) DC. var. *pusillum* (Massal.) Flag.). 48, Bala area, 1954, P. W. James. 49, dry limestone rocks, Little Orme, 1954, P. W. James. I have followed continental authorities in treating this as a distinct species.

Caloplaca cirrochroa (Ach.) Th. Fr. 49, limestone rocks near Little Orme, 1954, P. W. James.

Caloplaca variabilis (Pers.) Th. Fr. 64, limestone rocks, Fountains Fell, 1955, A. E. Wade. !

Physcia elaeina (Wahlenb.) A. L. Sm. 48, on elder, Harlech, 1923, D. A. Jones. ! On sycamore, Glanllyn, Bala, 1954, P. W. James.

Physcia wainioi Räs. 88, on sycamore, Inverhaggernie, Crianlarich, with *Parmelia tiliaceae*, *Lecanora rugosella*, *Physcia pulverulenta*, *P. ascendens* and *P. ciliata*, 1940, E. C. Wallace.

Physcia sciastra (Ach.) Du Rietz. 42, Cefn Cel Sanws, near Vaynor, 1932. !

Physcia dubia (Hoffm.) Lettau. New to Britain. 33, on a slate roof by a footpath to Bishop's Cleeve, 1922, H. H. Knight. ! 48, on slates of a barn, Bryn Mawr, Dolgelly, 1941, R. Burn.

P. dubia is easily overlooked on account of its superficial resemblance to some forms of *P. tenella* and *P. caesia*. The absence of marginal cilia, however, readily separates it from the former. From *P. caesia* it may be distinguished by the absence of the subglobose soralia characteristic of that lichen, and from that species and *P. teretiuscula* by the negative reaction of the medulla to K. It differs from *P. tribacia* with which it has been confused on the Continent, in the darker grey colour of the thallus, in the irregularly furcate or pinnatifid apices of the lobes, and in the lower cortex not being cellular. Both *P. dubia* and *P. tribacia* have marginal soralia, which in the former are labriform, but not so in the latter. *P. dubia* is usually saxicolous, but has been found on trees and palings on the Continent. The chemical reactions are cortex K⁺ yellow, Pd⁺ slowly yellow; medulla K⁻ Pd⁻.

Rhizocarpon geographicum (L.) DC. The majority of specimens in the Welsh National Herbarium from localities throughout the British Isles give a reaction of Pd⁺ orange, but the following have a negative reaction and come under *R. riparium* Räs.

34, on quartz, Buckstone, 1919, H. H. Knight. 35, on millstone grit, Mynydd Machen, 1934. 41, on Pennant grit, below Craig-y-llyn, and Mynydd Rudry, 1932: on millstone grit, Caerphilly Common, 1934. 42, on sandstone, near Craig-y-cilau, 1932; on quartz, near Ystradfellte, 1934. 60, on coarse grit, Tarnbrook Fell, 1902, J. A. Wheldon and A. Wilson; Hill Crag, Wyresdale, 1906, J. A. Wheldon and A. Wilson. 71, Slien Whallin, 1908, ? collector. 88, Ben Chalum, 1910, J. A. Wheldon and A. Wilson. 97, Ben Dothaid, 1910, J. A. Wheldon and A. Wilson.

Since there does not appear to be any morphological character associated

with the negative reaction to Pd *R. riparium* is better treated as a chemical form of *R. geographicum*.

Rhizocarpon reductum Th. Fr. The following new county records are represented by specimens in the Welsh National Herbarium: 9, 10, 11, 15, 33, 46, 57, 59, 60, 71, 98, 103, 107, 109, 111, 112, 116, 121, and the Channel Islands.

Cladonia tenuis (Floerke) Harm. 64, Helwith Moss, near Horton in Ribblesdale, 1955, J. R. Laundon.

Cladonia mitis Sands. 64, Malham Moss, Malham Tarn, and Fountains Fell, 1955, J. R. Laundon and A. E. Wade. !

Cladonia crispata (Ach.) Flot. var. *infundibulifera* (Schaer.) Wain. 41, Rudry Common, 1955. ! var. *gracilescens* (Rabenh.) Wain. 35, Taren-yresgob, Llanthony Valley, 1944, R. Lewis. ! 41, Craig Fach, near Cwmparc, 1952. ! 42, by Llyn-y-fan-fawr, 1927. ! Near Ystradfellte, 1935. ! 60, Greygarth Fell, 1906, J. A. Wheldon and A. Wilson. ! 64, Tarn Moss, Malham Tarn, and Helwith Moss, near Horton in Ribblesdale, 1955, J. R. Laundon and A. E. Wade. !

Cladonia furcata (Huds.) Schrad. 45, between Carn Llidi and St Davids, 1954, Mary Barnes. !

Cladonia squamosa (Scop.) Hoffm. var. *denticollis* (Hoffm.) Floerke. 64, Tarn Moss, Malham Tarn, 1955, J. R. Laundon and A. E. Wade.

Cladonia macilenta Hoffm. var. *scabrosa* (Mudd.) Cromb. 64, Tarn Moss, Malham Tarn, 1955, J. R. Laundon and A. E. Wade.

Cladonia bacillaris Nyl. 64, Helwith Moss, near Horton in Ribblesdale, 1955, J. R. Laundon and A. E. Wade.

Crocynia aeruginosa (Schaer.) Hue. 64, shaded tree trunks about Malham Tarn, 1955.

Peltigera pulverulenta (Tayl.) Nyl. (*Peltidea pulverulenta* Tayl., 1847). J. W. Thomson (1955) shows that this name takes precedence over *Peltigera scabrosa* Th. Fr. 1860.

Roccella fuciformis (L.) DC. 45, Skokholm Island, 1953, Donald Pigott. This is the first record for Wales.

Opegrapha saxicola Ach. 52, Rhoscolyn, 1954, P. W. James.

Opegrapha caesariensis Nyl. 52, Rhoscolyn, 1954, P. W. James. !

Verrucaria mucosa Wahlenb. 52, Rhoscolyn, 1954, P. W. James.

Verrucaria prominula Nyl. var. *minor* A. L. Sm. 2, on shale, near St Austell, 1953, P. A. Grundy.

Polyblastia schraderi (Sm.) A. L. Sm. 64, limestone pavement, High Folds, Malham Moor; limestone rocks, Fountains Fell, and Dry Valley above Malham Cove, 1955. !

Porina leptalea (Dur. & Mont.) A. L. Sm. 42, on birch, Mellte Valley, 1955, P. A. Grundy.

I am deeply indebted to the Director and Dr Ilmari Hiitonen of the Botanical Museum, University of Helsinki, for the loan of specimens of *Lecanora conizaea*, and to Dr W. Watson for the loan of the Dolgelley specimen of *Physcia dubia*. Thanks are also due to Dr R. A. Maas Geesteranus, Mr D. Griffiths and Mr J. R. Laundon for their valued help.

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(Accepted for publication 30 January 1956)

PUCCINIA SONCHI ROB. & DESM.

By P. O'CONNOR

(With 5 Text-figures)

Evidence is presented that *Puccinia sonchi* Rob. & Desm. has been applied to two rusts, *P. sonchi* Rob. & Desm. and *Uromyces sonchi* Oud., for which emended descriptions are given.

Sixty-five gatherings were examined of plants of species of *Sonchus* affected with rusts having teleutospores and uredospores (Fig. 1) in paraphysate uredosori (Fig. 2). Fifty-seven of the collections were made in thirteen of the southern vice-counties of Ireland at stations not less than 1 km. apart; six came from four English vice-counties, one from Scotland and one from Wales. In only five of the collections were the rusts found to tally with the description of *P. sonchi* given by Roberge & Desmazières, *Ann. Sci. Nat.*, Ser. 3, 11, 274 (1849).

In forty-seven of the collections, twenty-six from an area of about 70 sq.km. around Oranmore, Co. Galway, all the teleutospores were seen to be 1-celled (Fig. 3); in fourteen all were 2-celled (Fig. 4).

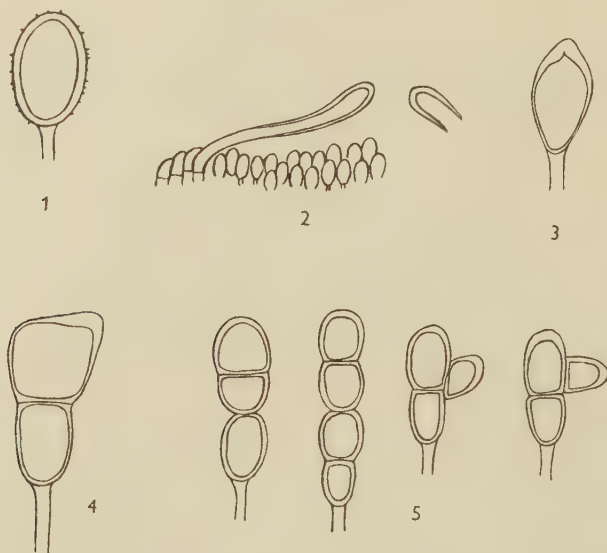
At one of the stations (in Co. Dublin) where mixed teleutospores were found, seventeen plants gathered from an area of about 6×1 m. were uniformly affected, the numbers of the 2-celled spores being in each case about five times those of the 1-celled spores; at a second station about 2 km. away, two plants were similarly affected and had the two kinds of spores in about the same relative proportions as to numbers; at a third station (in Co. Galway) the corresponding proportions between the two kinds of spores were seen to be reversed on a single plant; at a fourth station (in Wiltshire) on a single plant the 2-celled spores were about five times as numerous as the 1-celled ones.

Four gatherings of *P. sonchi* in the herbarium of the National Museum of Ireland were found to have mixed 2-celled and 1-celled teleutospores in about the following proportions as to numbers: Rostrup in Denmark 1874, 2.3:1; Jaap in Schleswig-Holstein 1904, 1.4:1; Jaap in Schleswig-Holstein 1904, 0.9:1; Jaap in Schleswig-Holstein 1908, 1.4:1.

At the Galway station, where mixed spores occurred, a subsequent search in the area of about 3000 sq.m. discovered twenty rusted plants of *Sonchus* all bearing uredospores in paraphysate uredosori and 1-celled teleutospores only.

There was not any significant difference observed between the unmixed 1-celled teleutospores which occurred alone and the 1-celled teleutospores or 'mesospores' which were found associated with 2-celled teleutospores; likewise, the unmixed 2-celled teleutospores resembled those which were found associated with 'mesospores' except for the occasional occurrence among the latter of 2-septate and 3-septate elements (Fig. 5).

The incidence of the teleutosori varied between abundance and scantiness; in the majority of cases, they were sufficient for ready detection with the unaided eye. In the greater number of gatherings, there was a gradual passage from uredosori to teleutosori areas. In eleven cases the occurrence of uredosori was sparse, consisting only of a few pustules among the teleuto-



Figs. 1-5. *Puccinia sonchi* and *Uromyces sonchi*. Fig. 1. Uredospore. Fig. 2. Uredosorus. Fig. 3. Teleutospore of *U. sonchi*. Figs. 4, 5. Teleutospores of *P. sonchi*.

sori. In some of these and in uredosori in transition zones of the remaining gatherings, uredospores were frequently found mixed with teleutospores in paraphysate sori.

Significant differences were not observed in either the uredospores or the associated structures in any of the three groups.

Seven seedlings of species of *Sonchus* affected with the uredospore stage of a paraphysate rust, were transplanted in the August-September period of 1954 from two districts in which the 1-celled teleutospore form of rust had previously been collected; eight similarly affected seedlings from stations which had produced 2-celled teleutospores were planted alternately with these, all neighbouring plants being spaced at distances of about 25 cm. apart. As well, seven similarly affected seedlings were transplanted in two groups, of each suspected constitution as to rust, similarly spaced at distances of about 8 m. on either side of the mixed plants. For a month at 3-day intervals the mixed seedlings in the central group were lightly brushed successively with a dry tuft of sterilized cotton-wool, with the object of transferring uredospores from plant to plant. At the end of the month, all the seedlings were covered with sheets of glass suitably supported at about 30 cm. above them. Flower-buds were removed as they appeared.

All the plants remained stunted in growth. The foliage of the cross-inoculated ones became abundantly covered with uredo-pustules in contrast with that of the more sparsely affected controls.

The plants were examined weekly *in situ*; teleutosori were not observed on any of them in growth. During September, October and early November, twelve of the cross-inoculated plants died; teleutospores were not seen on microscopic examination of the dead plants nor on any of the controls. At mid-November, the three cross-inoculated plants that remained alive were gathered. On two of these a few teleutosori were found containing in one case mixed teleutospores in the proportion of ten 2-celled to one 1-celled and in the other eight 2-celled to one 1-celled.

The grouping of all these forms under *P. sonchi* is not satisfactory; the classification as a *Puccinia* of a fungus characterized by 1-celled teleutospores is not acceptable. These forms may be arranged in two groups having respectively 1-celled and 2-celled teleutospores together with a third group in which the teleutospores consist of a mixture in varying proportions of 1-celled and 2-celled elements.

The occurrence of a form with 1-celled teleutospores over a wide area, the frequency of stations for it in one district that was systematically searched and the abundance of it at one of these stations appear to afford sufficient evidence to warrant the recognition of a species of *Uromyces* as described in part by Oudemans, *Hedwigia*, **11**, p. 181 (1872) on *Sonchus arvensis*. There seems to be no valid reason why the rejected *U. sonchi* Oudemans should not be revived and reinstated in a modified form with a uredo stage.

Two collections by Oudemans in the herbarium of the National Museum of Ireland were examined. In one (no. 1592) made at Amsterdam in 1871 and labelled '*Uromyces sonchi*', a few uredospores were seen in teleutosori. In the other labelled 'Fungi Neerlandici, no. 137. In fol. *S. arvensis*. *Puccinia sonchi* Rob. teleutosp. (sporis 1-locul.) Oct. 1875', a few paraphysate uredosori containing uredospores were seen among the teleutosori.

In view of the occurrence of unmixed 2-celled teleutospore forms at thirteen stations in eight vice-counties of Ireland and at one in England, there does not appear to be any reasonable doubt of the existence on species of *Sonchus* of an ordinary *Puccinia* uncomplicated with 'mesospores'. It is proposed therefore to regard this form as a species of *Puccinia*.

While it is recognized that the slender experimental evidence afforded by the cultures here described is insufficient alone to prove the composite nature of *P. sonchi* Rob. & Desm., the evidence gives reasonable support to that conception; and the conception appears, even without that support to be rational enough to warrant adoption pending a clearer exposition of the position.

If these conclusions are valid, then *P. sonchi* Rob. & Desm. may be regarded as a conerescent mixture of the *Puccinia* and the *Uromyces* now envisaged.

The following modified descriptions are offered:

Uromyces sonchi Oud. emend. O'Connor

Soris uredosporiferis primo epidermide bullato-vesiculosa obtectis, dein ab ea cupulari-forme-cinctis, rotundato-pulvinatis, sparsis vel nullo ordine aggregatis; peridiis muris 2-4 cel. crassis; paraphysibus 1-seriatis apice clavatis, $40-140 \times 15-20 \mu$, brunneis; uredosporis globosis, ovatis, ellipticis aut oblongis, $25-36 \times 14-21 \mu$, verrucosis, episporio crasso hyalino, contentu hyalino vel flavo-oleagineo, pedicellatis ad 36μ longis; soris teleutosporiferis sparsis vel aggregatis, magis compactis, saepe confluentibus, atris, nunquam pulveraceae, epidermide folii vel caulis semper tectae; teleutosporis in peridiis muris 2-4 cel. crassis dense stipitatis, pedicellatis ad 36μ longis, unilocularis, ovatis, clavatis, oblongis vel cuneatis, apicis saepius processis mamilliformis praeditis ad 7μ crassis, $40-60 \times 18-25 \mu$, fuscentis.

In foliis caulibusque Sonchi oleracei apud Glandorem in Hibernia.

Puccinia sonchi Rob. & Desm. emend. O'Connor

Soris uredosporiferis primo epidermide bullato-vesiculosa obtectis, dein ab ea cupulari-forme-cinctis, rotundato-pulvinatis, sparsis vel nullo ordine aggregatis; peridiis muris 2-4 cel. crassis; paraphysibus 1-seriatis apice clavatis, $40-140 \times 15-20 \mu$, brunneis; uredosporis globosis, ovatis, ellipticis aut oblongis, $25-36 \times 14-21 \mu$, verrucosis, episporio crasso hyalino, contentu hyalino vel flavo-oleagineo, pedicellatis ad 36μ longis; soris teleutosporiferis sparsis vel aggregatis, magis compactis saepe confluentibus, atris nunquam pulveraceae, epidermide folii vel caulis semper tectae; teleutosporis in peridiis muris 2-4 cel. crassis, dense stipitatis, pedicellatis ad 36μ longis, bilocularis ellipsoideis, oblongis vel cuneatis, medio constrictulis, basi rotundatis vel in pedicellum angustatis, apice incrassatis ad 7μ , rotundatisque, $30-60 \times 18-30 \mu$, brunneis.

In foliis caulibusque Sonchi oleracei apud Leapem in Hibernia.

This piece of work owes much to generous help gratefully received during its progress. Sincere thanks are offered to Dr R. W. G. Dennis of Kew for assistance and advice; to Dr G. R. Bisby of the Commonwealth Mycological Institute for advice and help with the diagnoses; to Miss Beatrice Salkeld (now Mrs Behan) of the National Museum, Dublin, for help with the cultures and inoculations; to Mrs Ida Reed, of Norton St Philip, Somerset, for specimens from Somerset and Wiltshire; to Mr M. Parker, B.Sc., of Cambridge for specimens from his neighbourhood, from Dorset and from Midlothian; to Mr E. J. O'Donovan, N.T., of Union Hall, Co. Cork, for rusted seedlings of *Sonchus* from his area and to Dr John O'Connor of Oranmore, Co. Galway, for an extensive and excellent collection of rusted plants diligently and systematically made over 70 sq.km. of his district and as well for conducting a duplicate set of experimental cultures which however gave no help owing to failure to produce observed teleutospores and, also, for the drawings here reproduced.

The relevant specimens are deposited in the herbarium of the National Museum of Ireland at Dublin.

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PATCH YELLOW DISEASE OF THE OIL PALM

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(With Plate 16 and 1 Text-figure)

The symptoms of Patch Yellow are described. Inoculation experiments are reported which demonstrated that *Fusarium oxysporum* is the causal agent and that the susceptibility to the disease varies according to the age of the palm.

Patch Yellow is a conspicuous foliar disease in oil palm (*Elaeis guineensis*) plantations in the Belgian Congo. Wardlaw (1946) isolated a strain of *Fusarium oxysporum* from infected palms, and this fungus was fully described by Gogoi (1949). Bull (1954) reported the isolation of *F. solani* and *F. moniliforme* from Patch Yellow palms in Nigeria and considered that the disease may be caused by several *Fusarium* spp. There is no record in the available literature of the pathogenicity of any fungus being proved for this disease.

In the Belgian Congo, the disease has been observed in all the oil palm plantations visited. Two-year-old palms may show symptoms of the disease but it is more severe in palms of 6 years and older. The number of palms infected with Patch Yellow varies between 0.2 and 1.8 % of the total, and it is generally considered that the majority of oil palms are resistant to the disease.

SYMPTOMS OF THE DISEASE

There is a complete range of disease symptoms from a very mild condition to an extremely serious condition, apparently depending on the degree of susceptibility of the infected palm. Thus, mature, infected palms show little change in the degree of infection, and mildly infected, adult palms have not been observed to develop severe symptoms at a later date. Palms do not recover from Patch Yellow, but the disease is not fatal and infected palms may continue to produce fruit bunches.

Infection occurs in the young, closed 'spear' leaves. With a mild infection, a faint brown speckling may be observed, in localized patches, on the folded leaflets. The patches are more extensive on the distal leaflets and at the distal end of the leaflets. The speckling spreads inwards from the edges towards the central nerve of the leaflets. The infection is contagious and spreads from one half of the folded lamina to the other half, as well as to adjacent leaflets which are in contact with the diseased patches. Thus, when the leaflets unfold the disease pattern is, approximately, symmetrical. With the opening of the leaf, chlorophyll fails to develop in the infected parts and this results in the appearance of yellow patches which are responsible for the name given to the disease. The progress of the disease is arrested with the opening of the leaves.

More severely infected palms show a more well-defined speckling of the

closed leaflets with the individual disease spots being larger and a darker brown colour. The yellow patches on the opened leaves are more conspicuous whilst closer inspection shows the presence of orange and brown, necrosed spots in the yellow patches.

With still more heavily infected palms the speckling is largely replaced by a rotting of the folded leaflets. The rotted tissue is a dark brown, nearly black, colour, and is moist and zonated. A zone of speckling may or may not be present around the border of the rotted tissue. Parts infected immediately before the opening of the leaves do not become rotted but remain speckled. With the opening of the leaves the rotted areas become desiccated, whilst the speckled areas and the zone bordering the rotted areas show the typical yellow colour associated with a mild infection. The desiccated, rotted parts of the lamina tend to break away with the ageing of the leaf and this results in the leaflets having a 'bitten' appearance (Pl. 16, fig. 2). Conidiophores and conidia of a *Fusarium* sp. are associated with the early stages of the leaflet rot, but older rotted tissues may show the presence of *Helminthosporium* spp. including *H. stilbaceum*.

Although Patch Yellow is a foliar disease, severe infection may retard the growth of the roots (Kovachich, 1952), and the poor root development results in the reduced growth of the stem and an abnormal habit of the leaves (Pl. 16, fig. 1). Thus, badly infected palms have attenuated trunks and abnormally erect older leaves so that the general aspect resembles that of Plant Failure palms.

When palms of 2-3 years of age are infected, rotting of the leaflets is not usually associated with the disease, and the yellowing is in the form of a speckling, with the individual infection spots retaining their separate identity, even in the older leaves. The symmetrical arrangement of the yellow patches is similar to that found in mature, infected palms.

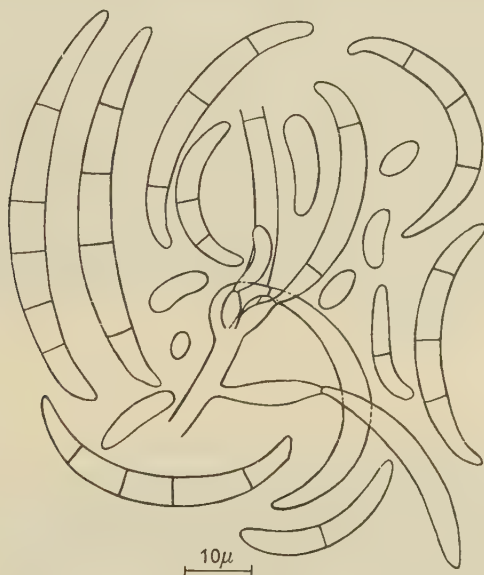
PROOF OF PATHOGENICITY

Cultures of diseased tissue from the folded leaflets of Patch Yellow palms consistently resulted in the isolation of a *Fusarium* sp. similar to that associated with the rotting of the leaflets. This fungus has been identified by Dr Gordon, as *F. oxysporum* Schlecht. ex Fr. No other *Fusarium* sp. has been isolated from the disease during this investigation.

During 1950, an inoculation experiment was laid down at Brabantia in the southern region of the Belgian Congo. This experiment included sixteen plots, each plot containing five, 6-year-old palms. The central spears of the palms in eight of these plots were sprayed with a 7-day-old, single spore culture of *F. oxysporum*, grown on a 2.5 % malt-extract solution. The inoculated fungus was originally isolated from a severely infected palm. The palms of the control plots were sprayed with a similar, sterile solution. Three of the inoculated palms showed typical symptoms of a mild infection of Patch Yellow 4 months later and the inoculated fungus was re-isolated from these palms. The experiment was repeated during 1952 on eighty 2-year-old palms at Yaligimba and resulted in nine of the forty inoculated palms showing symptoms of the disease 6 weeks after the inoculation. *F. oxysporum* was again re-isolated from the infected palms.

THE CAUSAL ORGANISM

F. oxysporum from Patch Yellow palms grew well on malt agar, corn meal agar, prune agar and Gogoi's modified Brown's agar. It was tolerant to a wide pH range, showing only slight differences in growth between pH 5.6 and 7.6. In general the cultural behaviour of the fungus agreed with that reported by Gogoi. Three-week-old cultures on Gogoi's medium showed a predominance of 3-septate macroconidia which measured $20-40 \times 3.8-4.2 \mu$. The microconidia measured $4.5-18 \times 3-5 \mu$. The conidia asso-



Text-fig. 1. *Fusarium oxysporum* from folded leaves showing symptoms of Patch Yellow.

ciated with the rotted leaflets were 0- to 5-septate with the 3-septate macroconidia measuring $19-42 \mu$ whilst the 5-septate conidia were, occasionally, 60μ long. The latter macroconidia were more abundant on the rotted leaves than in cultures of the fungus.

Microconidia were abstricted from short, lateral conidiophores or directly from the mycelium, whilst macroconidia were produced from lateral conidiophores which occurred in groups on the infected tissue (Text-fig. 1).

SUSCEPTIBILITY TO THE DISEASE

The results of the inoculation experiments are in accordance with the view that only a limited number of palms are susceptible to the disease, although the relationship between susceptibility and genetical factors has not been demonstrated. As the *F. oxysporum* used in the inoculation experiment was isolated from a severely infected palm but was only capable of producing a mild infection in the experimental palms, it would appear that the intensity

of the disease depended here on the variable susceptibility of the host rather than on the existence of physiologic races of the pathogen.

The possibility of testing nursery palms for susceptibility to the disease was considered. Twenty palms, each of 3, 6, 12 and 18 months of age were inoculated to ascertain whether nursery tests would be effective. No infection resulted from the inoculation of 3, 6 and 12-month-old palms, whilst ten of the twenty 18-month-old palms became infected. From these results it seems that nursery palms are not susceptible to the disease and that the susceptibility varies with the age of the palm. When the results of all the inoculation experiments are considered it is found that no palms were susceptible under 1 year of age, 50 % were susceptible at 18 months, 22.5 % at 3 years and 7.5 % at 6 years of age. In plantations, it is rare to find more than 1.5 % of the palms infected, so that it appears that all susceptible palms are not infected. There is, therefore, the danger that susceptible palms may be used for the production of selected seed which would lead to an increase in the disease incidence in future plantations. It is, therefore, recommended that all palms selected for seed production should be tested for susceptibility to the disease by spraying the central 'spears' with a spore suspension of *F. oxysporum*.

The writer wishes to record his indebtedness to Huilever S.A. for permission to publish this report.

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EXPLANATION OF PLATE 16

Patch Yellow of Oil Palm

Fig. 1. General aspect of severely infected palm.

Fig. 2. Symptoms on fairly seriously infected leaflets.

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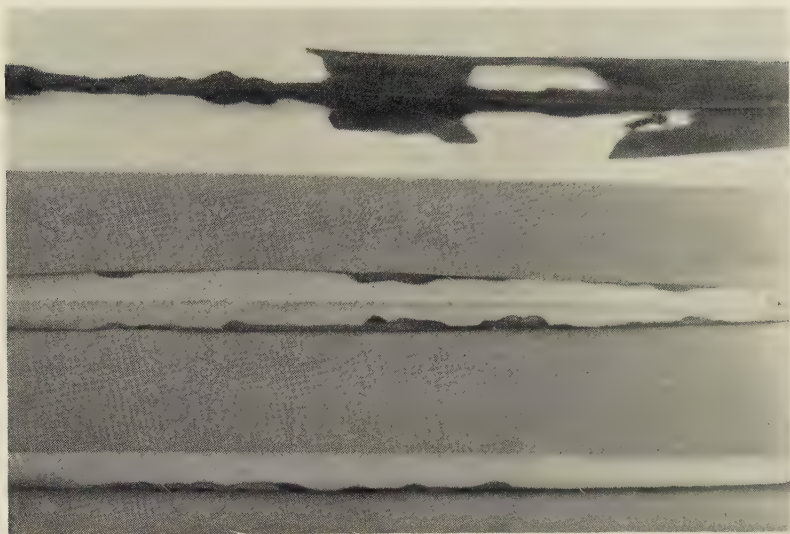


Fig. 2



Fig. 1

A CENSUS OF MOULD SPORES IN THE AIR OVER BRITAIN IN 1952

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(With 1 Text-figure)

From April to October 1952 Petri dishes containing Sabouraud's (maltose) agar were exposed out of doors under standard conditions at Cambridge, Cardiff, Edinburgh, Harpenden, Liverpool, London, Llandough, Southampton and York. About seventy genera of fungi were recorded but nearly 90% of the entire catch from all stations was made up by *Cladosporium*, *Pullularia*, *Penicillium*, *Epicoccum*, *Botrytis*, *Alternaria*, Sphaeropsidales, *Aspergillus* and *Sporotrichum*. *Cladosporium* was overwhelmingly predominant. Stations showed no differences one from another in the identity of the moulds caught, but *Cladosporium*, *Epicoccum*, *Botrytis*, *Alternaria* and *Pullularia* were relatively more abundant in the rural areas than within the cities, whereas *Penicillium* and *Aspergillus* were more abundant in the centres of London, Liverpool and Cardiff than at the less urban stations. There was a marked *Cladosporium* 'season' extending over the same period—June to September—at all stations.

INTRODUCTION

During the last two decades doctors have shown an increasing interest in the fungal spores which pervade the atmosphere. Surveys of the mould spore content of the air in particular have become an essential part of the comprehensive study of respiratory allergy. In Britain the earliest investigations of atmospheric mould spores from the point of view of allergy were made by Hyde & Williams using at first the adhesive-slide method (1946) and later the culture-plate method (1949). In continuing at this hospital during 1951 the series of Petri dish exposures begun at Llandough Hospital (3 miles away) by Hyde and Williams in 1948, it was observed that the catches in Cardiff showed distinct quantitative differences from the results of simultaneous similar exposures at Llandough and also from the earlier Hyde & Williams exposures at Llandough. At the latter site the total mould catch was higher with more *Cladosporium*, *Epicoccum* and *Alternaria*, and less *Penicillium* than in Cardiff. At that time Maunsell's (1952) study, using the slit sampler in London, was the only published investigation of atmospheric mould spores other than from Llandough. For these reasons a scheme was devised whereby the atmospheric moulds could be sampled simultaneously at nine stations in Britain.

The culture-plate method was chosen because notwithstanding its low sampling efficiency it was the only method which could be used on a large scale, and which would differentiate satisfactorily between most of the

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atmospheric moulds. People at six centres agreed to expose Petri dishes in collaboration with this Unit. These centres were the Botany School, Cambridge; the Department of Zoology, Edinburgh; the Chest Hospital, Liverpool; the Institute of Laryngology and Otology, London; Llandough Hospital; and the Department of Botany, Southampton. The number of stations were increased to a total of nine (including the base at Cardiff) by the generous co-operation of Dr P. H. Gregory and Mr Edward Yuill who carried out parallel investigations at Rothamsted Experimental Station and at Acomb, York, respectively, using material and techniques identical with those used at the other stations.

EXPERIMENTAL PROCEDURE

Early in 1952 each station was visited and a site selected for the daily exposure of culture plates. A shelter of the Hyde & Williams (1945) pattern was erected at each site to contain the plates during exposure, and to provide some shelter from rain while allowing free access to the air. The stations at Cambridge, Edinburgh, Liverpool, Llandough, London and Southampton received prepared Petri dishes from Cardiff, and after exposure returned them to Cardiff for examination. The Petri dishes were poured with Sabouraud's (maltose) agar and sealed with adhesive paper not less than 5 days before they left Cardiff. For despatch the plates were packed in boxes of spindle shavings, two plates to each box. The boxes and their contents of shavings and wrappings were all frequently sterilized to prevent contamination of the plates on their journeys. At the exposing station the plates were unsealed immediately before use, then resealed and repacked in the box for their return to Cardiff.

The time chosen for the survey was from April to October 1952. This period was known from previous experience at Cardiff to include the 'mould season'—mainly June to September—when the catches were much higher than during the rest of the year, and was thought to be as long as we could reasonably expect without overtaking our volunteer helpers.

Thus at these nine stations every weekday at 10 a.m. a culture dish was exposed for 10 min. by stop-watch. At Cardiff the usual practice there of exposing two plates consecutively each for 10 min. was continued. For the length of time of this survey the maximum number of weekday exposures which could have been made at all stations was 1860. In fact 1780 plates were analysed. The missing 4% included breakages, missed exposures (holidays, etc.) and a few plates which were suspected of contamination and were discounted.

THE STATIONS

The Botany School, Cambridge stands at approximately 40 ft. alt., about a quarter mile south of the town centre. There were many gardens to the west; and south-west within a quarter mile the surrounding land is only very loosely built upon and includes many lawns, gardens and playing fields. Outside a radius of 1-1½ miles, the town is surrounded by large

I am indebted to Prof. G. E. Briggs and Dr W. J. Dowson for facilities for exposing plates, and to Mr H. T. E. Smith, Assistant County Agricultural Officer, for information on land utilization and crops.

agricultural areas. The shelter was located on the 60 ft. high flat roof exposed in all directions.

St David's Hospital, Cardiff (approximately 25 ft. alt.) lies in a densely built-over part of the city. To the north-east and north, ranging from $\frac{1}{4}$ to $\frac{3}{4}$ mile away are the parklands of the Castle and south $\frac{1}{2}$ to $\frac{3}{4}$ mile away is commonland. Immediately surrounding the hospital and east and west, stretching some distance (about 2 miles), are closely built houses and streets with little in the way of gardens or parks. The shelter was situated on a small flat roof about 40 ft. above ground-level, somewhat sheltered on the east and west sides by the nearby sloping roofs of the same hospital block.

I am indebted to Dr John Jones, Medical Superintendent, for facilities for exposing plates, and to Mr Lloyd Lewis, Assistant County Agricultural Officer, for information on land utilization and crops.

King's Buildings, Edinburgh (approximately 250 ft. alt.) lies on the outer edge of the city suburban area about 2 miles from the centre of the city with Blackford Hill immediately to the west and the lower slopes of the Braid Hills $\frac{1}{2}$ to $\frac{3}{4}$ mile to the south. To the north-east and beyond the hills to the west stretches the city of Edinburgh. The shelter was situated on the flat roof (24 ft. high), exposed on three sides but somewhat shielded by a taller block of the same building some 30 ft. to the north.

I am indebted to Prof. James Ritchie for facilities for exposing plates, and to Mr A. M. Calder, Senior Inspector, Department of Agriculture for Scotland, for information on land utilization and crops.

Rothamsted Experimental Station, Harpenden (approximately 430 ft. alt.) is completely surrounded by a large rural and agricultural area. The shelter was placed on a platform 40 ft. high, exposed in all directions, in a mast constructed of open steelwork situated away from all buildings in one of the fields of Rothamsted Experimental Station.

I am indebted to Prof. P. H. Gregory for carrying out the census at Rothamsted Experimental Station, and for the Rothamsted meteorological records, and to Mr J. C. M. Bearder, County Agricultural Officer, for information on land utilization and crops.

The Chest Hospital, Liverpool (approximately 150 ft. alt) is surrounded by the densely built-up areas of the city. For at least a mile in all directions and more than this to the north-east and south, the area is densely built over. There are very few gardens or parks and no green areas more than a few square yards could be found in the vicinity of the hospital. To the west lies the Mersey and Birkenhead. The shelter was situated on a 31 ft. high flat roof protected to the north by a taller block of the same building, and there were some tall blocks of buildings some 50–100 yd. to the east. In the other directions the shelter was freely exposed.

I am indebted to Dr Blair Macauley and Dr J. Carr Brundrett for facilities for exposing plates, and to Mr A. H. Fitton, County Agricultural Officer, for information on land utilization and crops.

The Hospital, Llandough (approximately 200 ft. alt.) is situated in rural surroundings with woods and fields on all sides. To the north and west is considerable woodland with scattered groups of fields. To the north-east

1½–2 miles away, is the outer edge of Cardiff. To the east and south-east lies the sea coast mainly taken up with harbours and docks. To the south lies mainly cultivated fields. The shelter was located on a 22 ft. high flat roof freely exposed on three sides but shielded to the north by a taller block of the same building.

I am indebted to Dr D. G. Morgan, Medical Superintendent, for facilities for exposing plates.

Ear, Nose and Throat Hospital, King's Cross, London (approximately 55 ft. alt.) is in central London. The surrounding areas are densely built over but there are some few lawns and gardens round about. About 1 mile to the west is Regent's Park. Hyde Park lies about 2 miles to the south-west. The shelter was located on the 45 ft. high flat roof and was exposed in all directions.

I am indebted to Dr H. A. Lucas and Dr Kate Maunsell for facilities for exposing plates, and to Mr N. J. Wood, County Agricultural Officer, for information on land utilization and crops.

Department of Botany, University of Southampton (approximately 130 ft. alt.) lies to the north of the town centre in the outer suburban area. To the south lies the heavily built-up area of the town, stretching to the sea-front some 2 miles away. To the east and west the area is less densely built over, with gardens and wasteland to the west, for about 1 mile opening out into rural Hampshire, and with Southampton common (½ sq. mile in area) ¼ mile to the west. To the north lies a narrow band ½ mile of suburban development and beyond lie woods and fields for some miles. The flat roof of the Department of Engineering (about 30 ft. high), on which the shelter was situated, was exposed in all directions.

I am indebted to Prof. W. T. Williams and Dr J. G. Manners for facilities for exposing plates, and to Mr J. C. Clifford, District Agricultural Advisory Officer, for information on land utilization and crops.

Askham Lane, Acomb, York (approximately 85 ft. alt.) lies about 2 miles to the west of the Minster, on the edge of the built-up area. To the north, west and south the surrounding country consisted of cultivated fields, much under grazing but with some grain growing. A flat roof (some 12 ft. high) of a small private garage supported the shelter. The site was exposed in all directions, but a clump of tall trees about 20 yards to the south may have afforded some shielding.

I am indebted to Mr E. Yuill for carrying out the census at Acomb, York, to Dr H. Royle for help in arranging the scheme, and to Mr A. H. R. Lilly, Crop Husbandry Advisory Officer, for information on land utilization and crops.

RESULTS

Table 1 shows the composition of the catch of moulds at each of the nine stations. The same relatively few mould genera were abundant everywhere. The bulk of the catch at each station was made up by only eleven genera: *Cladosporium*, *Pullularia*, *Penicillium*, *Botrytis*, *Epicoccum*, *Aspergillus*, *Oospora*, *Candida*, *Alternaria*, *Sporotrichum* and *Phoma*. Other mould genera were caught only relatively infrequently or rarely (Table 2). The commonest

Table 1. *The commoner atmospheric moulds at nine stations in Britain in 1952*

	No. of colonies per 100 Petri dish exposures									Entire catch of all stations	Percentage of entire catch
	Cardiff	Cam-bridge	Edin-burgh	Harpen-den	Liver-pool	Llan-dough	London	South-ampton	York		
<i>Cladosporium</i>	649	2771	1132	5554	396	1340	890	1244	1386	29,213	69.6
<i>Pullularia</i>	116	175	121	113	105	68	170	103	211	2307	5.5
<i>Penicillium</i>	119	137	72	65	295	89	164	82	32	2078	4.9
<i>Epitoccum</i>	55	78	51	176	21	196	34	69	120	1537	3.7
<i>Mycelia sterilia</i> (white)	131	78	45	103	36	112	42	70	70	1478	3.5
<i>M. sterilia</i> (white and dark)	59	161	40	33	30	120	41	36	13	1056	2.5
<i>Botrytis</i>	33	49	75	80	38	49	31	48	80	919	2.2
<i>Alternaria</i>	24	91	3	29	12	15	27	35	17	493	1.2
Sphaeropsidales	35	20	21	6	44	20	50	19	13	466	1.1
<i>Aspergillus</i>	24	18	15	5	65	13	17	10	10	359	0.9
<i>Mycelia sterilia</i> (dark)	21	16	17	32	16	21	6	17	6	311	0.7
<i>Sporotrichum</i>	21	20	11	—	36	9	22	10	1	268	0.6
<i>Oospora</i>	20	21	10	169	20	24	6	8	2	544	1.3
<i>Candida</i>	21	23	7	7	11	13	16	13	—	236	0.6
<i>Monilia</i>	10	4	1	—	8	3	3	4	—	80	0.2
Other genera	44	60	20	47	31	22	39	25	29	639	1.5
Totals	1358	3722	1641	6419	1164	2114	1558	1793	1990	41,984	

mould at all stations was *Cladosporium* which alone constituted between 35% (at Liverpool) and 88% (at Harpenden) of the total catches over the whole period of the survey. *Penicillium* and *Pullularia* vied for second place in order of predominance at most stations.

Table 2. *The less common atmospheric moulds at nine stations*

(Among these the more frequently occurring are shown in bold type; the remainder occurred only rarely.)

PHYCOMYCETES	ASCOMYCETES	BASIDIOMYCETES
<i>Chaetocladium</i>	<i>Chaetomium</i>	<i>Fomes</i>
<i>Mucor</i>	<i>Eurotium</i>	(<i>Heterobasidion</i>)
<i>Pythium</i>	<i>Melanospora</i>	
<i>Rhizopus</i>	<i>Pleospora</i>	
<i>Syncephalastrum</i>		
FUNGI IMPERFECTI		
<i>Acremoniella</i>	<i>Gliocladium</i>	<i>Scopulariopsis</i>
<i>Acrospeira</i>	<i>Gliomastix</i>	<i>Sepedonium</i>
<i>Acremonium</i>	<i>Gonatobotryum</i>	<i>Spicaria</i>
<i>Acrostalagmus</i>	<i>Graphium</i>	<i>Spondylocadium</i>
<i>Acrothecium</i>	<i>Helminthosporium</i>	<i>Sporobolomyces</i>
<i>Allantospora</i>	<i>Hormiscium</i>	<i>Stachybotrys</i>
<i>Botryotrichum</i>	<i>Isaria</i>	<i>Stemphylium</i>
<i>Cephalosporium</i>	<i>Leptographium</i>	<i>Stilbella</i>
<i>Cercospora</i>	<i>Monotospora</i>	<i>Stysanus</i>
<i>Chloridium</i>	<i>Nematogonium</i>	<i>Tilletiopsis</i>
<i>Ciboria</i>	<i>Nigrospora</i>	<i>Torula</i>
<i>Curvularia</i>	<i>Oidiodendron</i>	<i>Trichaeogum</i>
<i>Dactylella</i>	<i>Pachytrichum</i>	<i>Trichoderma</i>
<i>Echinobotryum</i>	<i>Paecilomyces</i>	<i>Trichothecium</i>
<i>Fusarium</i>	<i>Papularia</i>	<i>Verticillium</i>

At Harpenden and Cambridge the total catches of moulds were much higher than at the remaining stations, especially London, Cardiff and Liverpool where the lowest catches were obtained. The largest variation among the stations was in the numbers caught of *Cladosporium*. The catch of this organism at Harpenden was fourteen times that at Liverpool, and the other stations ranged in between these two extremes. Details of the land utilization and crops within 1 and 3 miles of each station, obtained from the local agricultural officers, showed up some clear relationships. The numbers of *Cladosporium* caught were directly related to the area of open country and land under crops, especially cereals, within a few miles; or inversely related to the degree of urban development of the station's environs. *Alternaria*, *Epicoccum* and *Botrytis* were also more common at the more rural stations than in the large cities, but on the other hand, *Aspergillus*, *Sporotrichum* and in particular *Penicillium* were more common in the centre of the large cities than elsewhere.

The 'mould season' began at about the same time and continued for about the same period everywhere. *Cladosporium* is the main component of the summer rise, with much smaller but not insignificant contributions from *Pullularia*, *Epicoccum*, *Alternaria* and later *Botrytis* (Richards, 1954). In April and early May the numbers of *Cladosporium* caught are about the same low level at all stations, with only slightly higher catches at the stations with the greatest agricultural environs, Harpenden and Cambridge. When the *Cladosporium* season commences the uprush of numbers is much

greater at the rural stations than in the cities (Fig. 1). Thus during the height of the season the maximal atmospheric concentrations of spores (as indicated by Petri dish exposures) in the rural areas may average more than twenty times the concentration in the centres of the large cities.

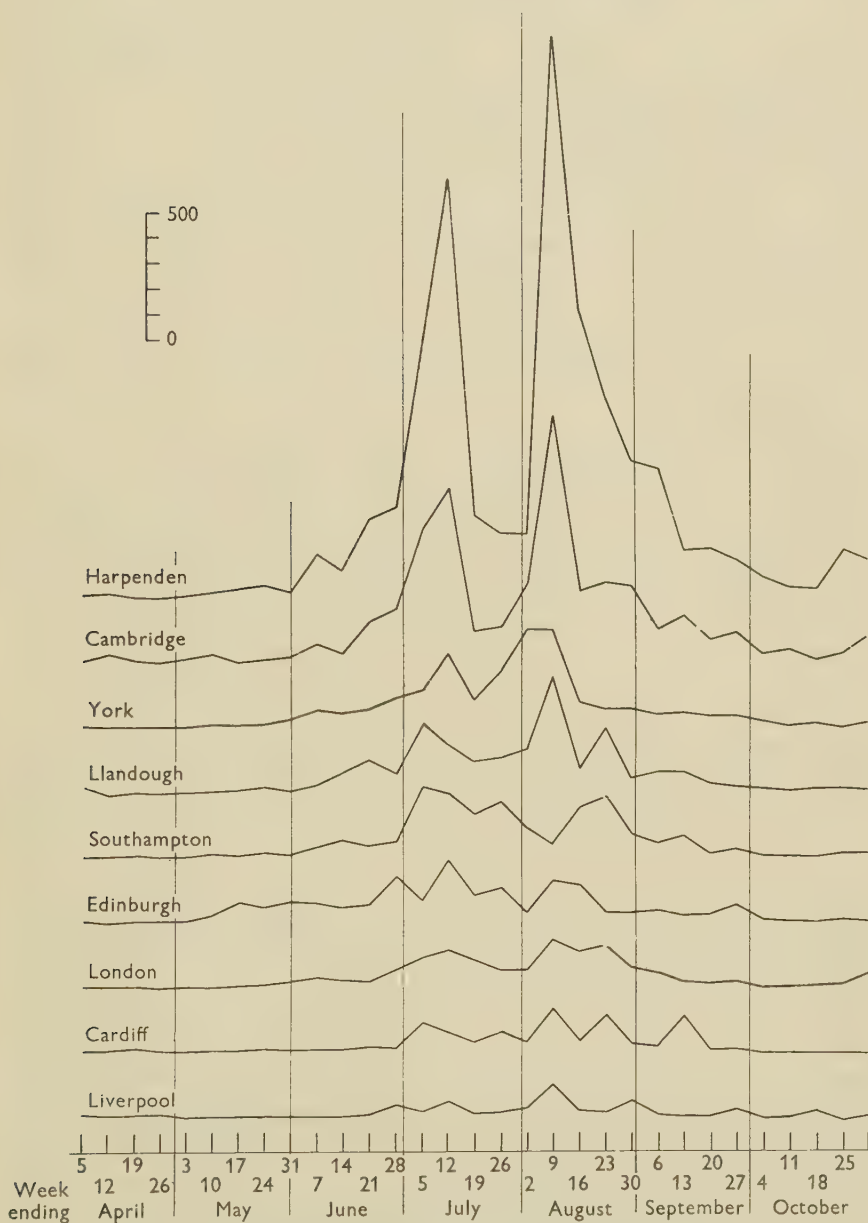


Fig. 1. Variation in weekly catch of *Cladosporium* at nine stations in Britain in 1952. (The first few points in each line are at or near 0.)

EFFECT OF WEATHER

Among the weather records* obtainable for nearly all our stations, rainfall showed the greatest variation from one station to another. Three times as much rain fell at Edinburgh and Liverpool than at Cambridge, London and York. The highest number of hours sunshine was recorded at Southampton, 25 % more than at Liverpool and York with the least sunshine. Highest mean daily maximum and minimum temperatures were recorded in London, and lowest in Edinburgh. Mean wind velocity appeared to be remarkably uniform among all stations.

None of these meteorological factors appeared to account for, or be related to, the variation in mould catch from one station to another. However, at any one station there is in general, as Hyde & Williams (1953) have pointed out, a broad correlation between the numbers of *Cladosporium* spores and the mean daily temperature.† High numbers of *Cladosporium* occur as a rule only when the mean temperature exceeds about 60° F. The correlation is by no means complete since the high catches are sometimes maintained in the face of a large drop in temperature, and similarly successive low catches can coincide with periods of high mean daily temperature.

A marked fall in the *Cladosporium* catches occurred suddenly and almost simultaneously at all stations in the period covered by the weeks ending on 12 July and 9 August. The counts fell to a level which was abnormally low for the time of the year and which was preceded and succeeded by the usual phenomenal high counts. The coincidence at nine stations of the marked fall in catch suggests the operation of a widespread factor, probably climatological. At all stations the reduction in catch coincided with an appreciable drop in mean temperatures. At Cardiff, Llandough and Southampton the fall in numbers occurred in the week previous to that of the remaining stations and so did the drop in temperature. (The same set of weather records was used for both Cardiff and Llandough.) By 26 July the mean temperatures had returned to the earlier high values, but the *Cladosporium* counts did not return to the previous heights until week ending 9 August (earlier at York, later at Southampton) by which time the

* The Meteorological Office of the Air Ministry kindly supplied the record sheets of daily observations at the seven meteorological stations nearest to our exposing sites, excepting Harpenden. Records for Harpenden were obtained from Rothamsted Experimental Station by courtesy of Dr P. H. Gregory. The nearest meteorological station to Llandough was the Cardiff station some 3 miles away.

Brief synopsis of the weather at the nine stations for the 31 weeks 1 April–1 November, 1952: *Mean weekly rainfall* (in inches): Liverpool 1.26, Edinburgh 1.25, Cardiff 0.95, Southampton 0.63, Harpenden 0.53, London and York 0.45, Cambridge 0.40. *Mean weekly number of hours of sunshine*: Southampton 40.3, Cardiff 38.5, Harpenden 38.2, Edinburgh 35.3, Liverpool and York 32.8. Not recorded at the London and Cambridge stations. *Mean daily maximum temperature* (° F.): London 67.0, Southampton 65.5, Cambridge 65.4, Harpenden 53.6, York, 63.2, Cardiff 62.2, Liverpool 59.2, Edinburgh 58.2. *Minimum*: London 50.8, Liverpool 49.2, Southampton 49.1, Cardiff 48.2, York, 48.0, Cambridge 47.0, Harpenden 46.7, Edinburgh 46.2.

† The average of the daily maximum and minimum temperature—the only measure of mean daily temperature to be derived from the daily record sheets of the meteorological offices.

mean temperatures had again fallen and were appreciably lower than in the preceding week or two.

The results seem to suggest, however, that a more accurate evaluation of mean daily temperature (combined perhaps with a sampling technique more efficient than the ordinary culture-plate method) might reveal the *Cladosporium* catch to be following quite closely—but with a time lag of some days—the mean temperature curve.

The other factor which might have had an effect during this period was rainfall. The 3 weeks ending 2 August, which covers the period of low *Cladosporium* catches at most stations, were remarkable for the paucity of rain which fell. There was no rain recorded for 17–18 consecutive days at most stations, and it is interesting to observe that at Edinburgh, York and Southampton where traces of rain occurred on several days the depression in the *Cladosporium* counts is not as clearly defined as at the stations where the dry period was apparently uninterrupted. This might suggest that absence of rainfall has reduced the numbers of *Cladosporium* spores in the air. Boughey (1949) points out that germination and production of mould conidia are favoured by free water or high humidity. However, Hyde & Williams (1953) have shown that much more *Cladosporium* was caught in the relatively dry summer 1949 than in the relatively wet summer of 1950.

These conclusions are not as conflicting as they might appear at first sight. Rainfall is likely to affect the atmospheric spora in several ways.

(1) Rainfall probably increases spore germination, mould colonization, mycelial growth and conidia production.

(2) It washes spores out of the air (Gregory, 1952) so decreasing atmospheric concentrations, and in source areas reducing the number of spores available for wide aerial dissemination.

(3) Heavy or prolonged rainfall may wash spores off conidiophores, reducing the number becoming airborne.

(4) Prolonged absence of rain, by killing crops, provides additional food material for mould colonization.

In determining the extent of these effects on the numbers of mould spores in the air, distribution of the rainfall in time is likely to be of more importance than the quantity of rainfall over a given period.

These considerations explain the relationship between the above observations of Boughey and Hyde & Williams, but only (1) would seem to indicate how the absence of rain might have helped to reduce the *Cladosporium* count during that period in July–August 1952. Clearly, the year during which subaerial spore production is highest is not necessarily the year when atmospheric concentrations of the spores are highest.

COMPARISON WITH OTHER EUROPEAN STUDIES

The results of this census are in close general agreement with studies made elsewhere in Europe.

Earlier in Britain, Hyde & Williams (1949) near Cardiff found *Cladosporium* making up 51 % of their year's catch, followed by *Penicillium* (13 %) and *Pullularia* (4 %). Maunsell (1952) reported *Penicillium* to be the most abundant airborne mould in the centre of London over the period January

to July 1951. Ainsworth (1952) found *Cladosporium* predominant both in the centre and in an outer suburb of London during a year's study 1947-8.

In Copenhagen, Rostrop (1908) and Flensburg & Samsoe-Jensen (1950) found *Cladosporium* to be the most prevalent mould caught on Petri dishes out of doors, making up half the entire mould catch. Next in order of abundance were *Penicillium* and *Pullularia*. At Orebro, Sweden, Nilsby (1949) found that *Cladosporium* made up 68 % of the entire mould catch again followed by *Penicillium* and *Pullularia*. In Paris, Vallery-Radot, Halpern, Secretain & Domart (1950) also reported *Cladosporium* to be the commonest airborne mould, but the next commonest was *Alternaria* followed by *Penicillium*. In Padua and Turin, Peyronel (1914) and Volterrani (1954), respectively, both reported *Cladosporium*, followed by *Epicoccum* and *Alternaria*, to be the predominant atmospheric moulds. At Madrid, Canto Borreguero & Jimenez-Diaz (1945) report *Penicillium*, *Alternaria* and *Cladosporium* to be the most prevalent airborne moulds. Similar results were obtained at Barcelona by Frouchtman (1946) and Alemany-Vall (1949), and also at Cadiz by Diaz-Rubio and collaborators (1950), but at Alcazar de San Juan (some 150 km. from Madrid) *Cladosporium* predominated in the survey by Morales Musulen & Canto Borreguero (1946). Thus, wherever studies have been made in Europe, the spores predominating in the air belong to the same few genera of moulds.

I am grateful to Dr D. A. Williams and Mr H. A. Hyde for much help and encouragement, and to the staff of the Asthma and Allergy Research Unit and of the various stations without whose assistance this work would have been impossible. This work was carried out during the tenure of a research grant from the Asthma Research Council, London.

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CULTURAL CHARACTERISTICS OF DYSGONIC STRAINS OF *MICROSPORUM CANIS* BODIN

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(With Plates 17 and 18, and 1 Text-figure)

The distinctive cultural features of dysgonic and eugonic strains of *Microsporum canis* are described. A study of the growth of both dysgonic and eugonic types, as derived from single spores, at various temperatures, showed that at 31° C. the colonies of dysgonic strains were equal in size to those of eugonic strains and the hyphal tips were eugonic in character. The growth of dysgonic strains was restricted to a narrow temperature range as compared with that of eugonic strains. The significance of these findings is discussed, and it is suggested that the dysgonic strains are degenerate forms of this species.

In Britain *Microsporum canis* is common and widespread as the cause of the animal-type microsporosis of man, acquired from cats and dogs. General and local surveys by Walker (1950*a*), Carlier (1954), Ainsworth & Austwick (1955), Miss Mary English (personal communication) and La Touche (1955) have made this clear. Cultural varieties of *M. canis*, distinguished by pigment formation on agar media, were described by Walker (1950*b*) as yellow, orange, citreous, or, if non-pigmented, as '*var. album*' [nom. nud.]. In addition, Walker (1950*b*) described certain strains as dysgonic, becoming eugonic on subculture, and others which died after producing a small growth on and around the implanted hair. The terms 'eugonic' and 'dysgonic' have been borrowed from bacteriological terminology by various authors, notably by Duncan as quoted by Walker (1950*a*), to denote normal and stunted growths, respectively, of certain strains of *M. audouini*. In Leeds, fifty-four out of 144 strains of *M. canis* isolated during 5½ years were dysgonic on Sabouraud test medium (glucose-peptone agar), on Sabouraud preservation medium (peptone agar) and on a medium containing 5 % (w/v) honey, 1 % (w/v) Oxoid peptone and 2 % (w/v) agar, with or without 20 units penicillin and 40 units streptomycin per ml. to inhibit bacterial growth.

The characteristics of eugonic strains in routine isolations

(*a*) *Macroscopic*. After implantation of infected hairs in the above media, growth at 27° C. was rapid, becoming visible after 2-3 days (strain Wagstaffe) and, after 7 days, reaching mean diameters of 29.2 mm.

(Sabouraud preservation medium), 26.7 mm. (Sabouraud test medium) and 25.6 mm. (honey-peptone agar). The superficial growth, at first radiating and pellucid, became more dense and indefinite because of the development of a greyish to white aerial mycelium, especially on sugar-containing media. Radiating furrows gradually appeared (Pl. 18, fig. 1). The surface became light buff in colour after 10 days and some strains produced a yellow-orange pigment, especially seen on the reverse of the colony, whilst others produced a pale yellow ('citreous') pigment. Pigment formation by certain strains was limited to growths on sugar-containing media.

(b) *Microscopic*. All strains were studied at 27° C. by the microculture method (La Touche, 1951), since modified by the use of 10 % (w/v) gelatin instead of 0.15 % (w/v) agar, to increase clarity and ease of manipulation, and the addition of 20 units penicillin with 40 units streptomycin per ml., to inhibit bacterial contaminants. Spores present on the short lengths of human or animal hair used as inocula germinated within 24 hr. and branching of the hyphae occurred after 2-3 days, the branches arising immediately proximal to the septa of the main hyphae (Pl. 17, fig. 3) and growing forwards at acute angles to the parent hyphae. The hyphal tips (Pl. 17, fig. 4) were uniform. Macroconidia appeared after 3-5 days, especially around the hair, being thick-walled with fine granulations on the surface, spindle-shaped, varying in size with the strain ($63 \times 12 \mu$, being the mean of twenty measurements for strain Wagstaffe) and later developing from two to five transverse septa.

The characteristics of dysgonic strains in routine isolations

(a) *Macroscopic*. After implantation of infected hairs in the same media as used for eugonic strains, growth at 27° C. was slow, the colonies reaching a maximum diameter of 5 mm. after 7-10 days (strain Hirst), the growth being mainly within the agar gel. The majority of strains formed a variable amount of yellow pigment, chiefly in the hair and its immediate vicinity and in some cases only detected after microscopical examination. Subcultures from colonies after this period frequently failed to grow. Occasionally, after 7-10 days, a sector of the mycelium grew out rapidly as a typical eugonic growth and sometimes even masked the original colony. This has been observed in only a small proportion of dysgonic strains, possibly owing to the limited number of hairs which can be cultured in routine tests.

(b) *Microscopic*. In microculture at 27° C., germination of spores occurred within 24 hr. and the tips of the hyphae showed after 3-5 days forked, tufted, or swollen ends in great variety (Pl. 17, figs. 1, 2). These irregular tips were a very constant feature of dysgonic strains and could be detected as terminal thickenings in agar slope cultures, using a $\times 10$ hand-lens. Branches were less frequent than in eugonic cultures and their tips also were somewhat irregular. Production of macroconidia was inconstant, varying with the strain from none to very many.

Experimental work was undertaken to determine the factors controlling the relation between dysgonic and eugonic growth, especially with regard

to dysgonic strains which showed instability. Because of irregular results obtained after seeding hair fragments containing large numbers of spores of *M. canis*, and the possibility of contamination by other fungi and by bacteria, a single-spore technique was used which gave at least 90 % germination and freedom from contamination.

METHODS

Collection of material from hairs. Infected hairs were collected from cats infected with *M. canis*, using a Wood's lamp to detect infection of individual hairs by the fluorescence test. As a minimum, twelve fluorescent hairs were teased out and scraped in sterile water in a hollow-ground slide, using a scalpel and forceps, the resulting spore suspension being collected with a pipette, checked microscopically and stored overnight at 0–4° C.

Isolation of single spores and inoculation of media. A technique was devised to permit of the rapid isolation and culture of 120 spores by one operator in 5 hr., based on the method of Johnstone (1953), with the following modifications:

(1) *Agar blocks*, 2.5 × 1.0 × 0.2 cm., were cast from a clear glucose agar (2.75 % (w/v) 'British Agar', 1.0 % (w/v) Lab-Lemco, 1.0 % (w/v) Oxoid peptone, 0.5 % (w/v) sodium chloride, in distilled water at pH 7.6, to which was added sterile glucose solution (40 %, w/v) to give a final concentration of 3 % (w/v) before use). Each block was pre-marked with locating pits for twelve spore isolations and was stored overnight, under sterile conditions, at 0–4° C.

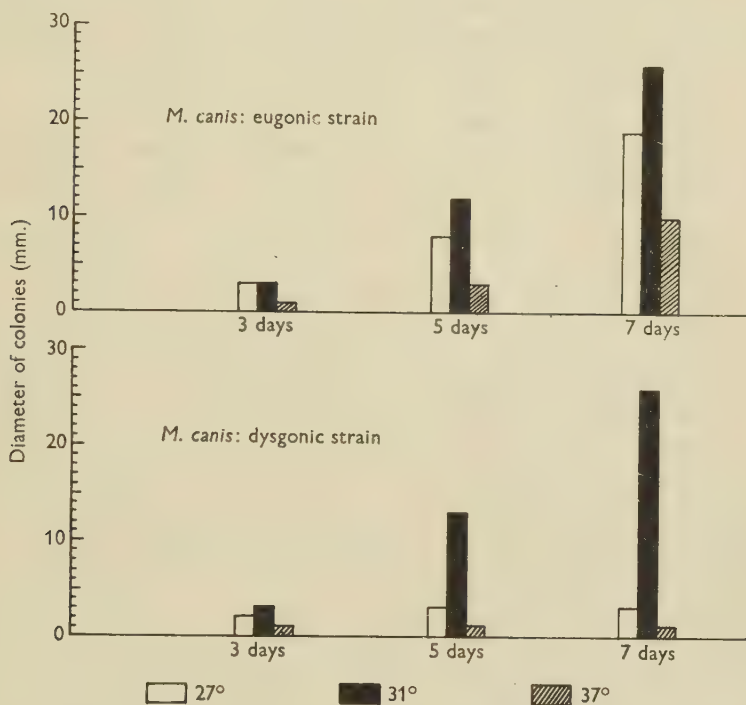
(2) *Phase-contrast illumination* was obtained by mounting a high-absorption phase plate (Beck) behind the back component of a 4 mm. Parachromatic objective (Watson), which gave a working distance of 1 mm. between cover-glass and agar block for the manipulations, a magnification of × 600 in the Bactil binocular microscope (Watson) with × 10 oculars, and excellent contrast. The spores, 2–4 μ in diameter, were seen as bright objects against the green background; bacteria and small particles of debris as intensely black objects. Only spores showing good contrast were selected, these being the most likely to prove viable.

(3) *A microloop* of soft glass made in the microforge was used to transfer the selected spores. It terminated in a filament 2.6 μ in diameter formed into a loop 15 × 17 μ, lying in the horizontal plane. On lowering the loop to surround the selected spore on the agar surface and again raising it, the spore was usually picked up with the water taken by the loop from the agar gel. After transfer to the selected site, the loop was lowered and the spore left on the agar surface, either by simple deposition, or by sinking the loop into the gel, which invariably left the spore on the surface, moving the agar laterally relative to the loop and then extracting the loop. Each transfer took approximately 1 min. and the same loop after being used for 294 transfers was undamaged.

The agar blocks were dissected and the small blocks, each carrying a single spore, were transferred to separate nutrient agar slopes containing 3.0 % (w/v) glucose, 20 units of penicillin and 40 units of streptomycin per ml., to be comparable with hair implantation experiments.

RESULTS

Germination occurred in approximately 90 % of spores isolated. The effect of the temperature of incubation on the subsequent growth of the colony was remarkable, and is shown in Text-fig. 1 in which the mean diameters of nine colonies of a eugonic strain and of a dysgonic strain are shown, at each of three temperatures. Throughout this work, diameters of colonies were measured along the lengths of slope cultures.



Text-fig. 1. *Microsporium canis*. The mean diameters of nine colonies of a eugonic strain (Wagstaffe) and of a dysgonic strain (Hirst), when incubated at 27, 31 and 37° C. for 3, 5 and 7 days. Single spore cultures, on agar blocks.

At 27° C. colonies of dysgonic strains reached a mean diameter of only 3 mm. after 7 days, this being the width of the agar block on which the spore was placed. In a few cases, as previously described, eugonic outgrowth took place, with rapid increase in size, reaching 19 mm. after 7 days. This was rare and erratic, occurring only once in 274 single spore cultures, but three times in a subsequent series of thirty-nine isolations made from the same cat (Hirst strain). Eugonic outgrowth is much more frequent when hair fragments, containing very large numbers of spores, are seeded into the medium.

The colonies of eugonic strains, on the other hand, showed a steady increase in size, passing beyond the margins of the agar block after 3 days

and reaching a mean diameter of 19 mm. after 7 days. This represents the diagnostic distinction between dysgonic and eugonic types, most routine work having been carried out at 27° C., but using hair implantation cultures.

At 31° C., however, dysgonic and eugonic strains grew equally rapidly, reaching mean colony diameters of 26 mm. after 7 days, but by 14 days, when the diameter of the colonies was approximately 50 mm., the superficial mycelium of the eugonic strains could readily be distinguished by its greater density and whiteness. These findings were confirmed for seven dysgonic strains of *M. canis* by seeding portions of infected hairs directly into glucose nutrient agar, and into agar blocks mounted on this medium, when results comparable with those obtained from single spores were found. Owing to the necessity of washing the hairs with 70% ethyl alcohol to reduce the risk of fungal contamination, and owing to the great variation in the number of viable spores seeded, the results were much less consistent than those from single spores, some hair fragments yielding no growth.

At 37° C. growth of dysgonic strains was stunted, the mean diameter of the colonies being only 1 mm. after 7 days, whilst microscopically the mycelium was very irregular with many vesicular chlamydospores. In two of thirty-four viable dysgonic single-spore cultures, typical eugonic outgrowth occurred very late, the colony reaching a diameter of 19 mm. after 15 days in one and 22 mm. after 31 days in the other. This was confirmed by hair implantation experiments. The eugonic strains grew steadily, but much less rapidly than at 31° C. reaching a mean diameter of 10 mm. after 7 days.

To facilitate photography, pairs of agar blocks, carrying single spores of dysgonic and eugonic strains, respectively, were placed on plates of glucose nutrient agar, in triplicate, and incubated at 27, 31 and 37° C. for 6 days (see Pl. 18, fig. 2):

To investigate the permanence of this temperature effect on dysgonic strains, ten cultures grown from single spores at 37° C. for 8 days, with a mean colony diameter of 1 mm., were transferred to 31° C. and observed for a further 12 days. Two days after transfer, no increase in size was visible, but on the third day the mean diameter was 6 mm., increasing to 43 mm. on the 12th day, with uniform hyphal tips and subsequent formation of a few macroconidia. On the other hand, ten cultures transferred after 8 days at 27° C. with a mean colony diameter of 4 mm., showed no change in size after 12 days at 31° C., the hyphal tips being of the typical dysgonic type and apparently incapable of further growth. Confirmation of the importance of temperature for the growth of dysgonic strains was found by making first subcultures from primary cultures of strain Hirst, grown for 28 days at 31° C., to glucose nutrient agar, incubating one series of the subcultures at 27° C. and the remainder at 31° C. as controls. At 27° C., a stunted, submerged mycelium with many irregular hyphal tips was obtained, whereas at 31° C., growth with regular tips and aerial mycelium occurred. The aerial mycelium never reached the abundance obtained with eugonic strains.

Growth at low temperatures. In view of the marked sensitivity of dysgonic

strains to a temperature variation of only 4° C. below, or of 6° C. above the optimum for their growth and the effect on the hyphal tips, the characters of the growths of dysgonic and eugonic strains were investigated by hair implantation in glucose nutrient agar maintained at $12.8 \pm 1.7^\circ \text{C}$. (Facilities for accurate thermostatic control were not available.) The effect on dysgonic strains was even more marked than at 27° C., the growth in microcultures being stunted, with much greater irregularities in the hyphae. Agar slope cultures showed scarcely perceptible growth to the unaided eye and a stunted, irregular growth microscopically, after 23 days. The eugonic strains in microculture showed a very retarded growth, with many irregular hyphal forms, including pectinate structures and chlamydospores, normal hyphae being scanty. In slope cultures, growth was slow, progressing to a mean colony diameter of only 27 mm. after 23 days.

Dysgonic strains are therefore restricted to a narrow temperature range above and below 31° C. for vigorous growth, whereas eugonic strains reach a good colony size over the wide range from 12.8 to 37° C., although growth is slow at the upper and lower limits of the range.

DISCUSSION

Dysgonic strains of *M. canis* appear to be degenerate forms of this species, as shown by (a) their limited growth and short survival at temperatures which are commonly used for the culture of dermatophytes, and which enable normal growth of typical (or eugonic) strains of *M. canis*; (b) the production of stunted hyphae with abnormal terminal outgrowths, as compared with the long and uniform tips of eugonic strains under the same cultural conditions. Sabouraud (1910) described similar abnormal hyphae in a strain of *M. canis* (*M. villosum* Minne), but which differed from the dysgonic strains here described in that the colonies were of normal size when cultivated at room temperature. He described these abnormal hyphae as 'organes de souffrance', thus signifying their unfavourable response to the cultural conditions. This view is entirely in agreement with the characteristics of the dysgonic strains described and with the fact that a rise in temperature of only 4° C. enables their hyphae to develop normally, so as to be indistinguishable microscopically from those of eugonic strains at the same temperature (31° C.). Walker (1950*b*) obtained increased aerial growth at 26° C. in dysgonic strains of *M. audouini* in culture medium to which had been added vitamins of the B group.

Dysgonic strains of *M. canis* are also in many cases unstable (Walker, 1950*b*) and evidence for this is provided by the outgrowth of eugonic sectors in many cultures from infected hair, or by the eugonic growth which occurred in a small proportion of single spore cultures. These facts suggest that dysgonic strains of *M. canis* were originally eugonic, but, owing to their prolonged parasitic existence at temperatures prevalent in the mammalian skin, have lost the power to adapt themselves to a saprophytic life at lower temperatures, the eugonic sectors which appear in cultures representing survival elements of the original eugonic growth. Since the habitat of these strains in the active growth phase appears to be restricted to animals and

man, it is suggested that eradication from these hosts would eliminate dysgonic strains as pathogens.

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EXPLANATION OF PLATES 17 AND 18

PLATE 17

- Fig. 1. *Microsporum canis*. Terminal irregularities of dysgonic hyphae (strain Hall). Microculture: 4 days at 27° C. (× 160.)
- Fig. 2. *M. canis*. Hypha of dysgonic strain Hirst, showing terminal tuft. Microculture: 3 days at 27° C. (× 412.)
- Fig. 3. *M. canis*. Hypha of eugonic strain Oddy, showing septa and characteristic branching. Microculture: 4 days at 27° C. (× 412.)
- Fig. 4. *M. canis*. Mycelium of eugonic strain Wagstaffe, showing uniform hyphal tips and branches. Microculture: 4 days at 27° C. (Phase contrast: × 228.)

PLATE 18

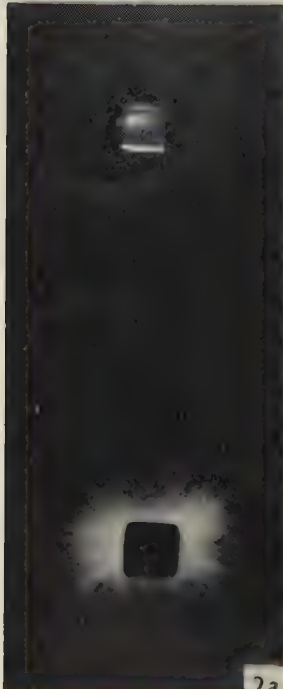
- Fig. 1. *M. canis*. A typical colony of a eugonic strain (Wagstaffe) on Sabouraud preservation medium after 18 days at 27° C. (× 1·5.)
- Fig. 2. *M. canis*. Single spore cultures on glucose nutrient agar blocks of dysgonic strain Hirst (above) and eugonic strain Wagstaffe (below), after 6 days at (a) 27° C.; (b) 31° C.; (c) 37° C. (× 2·0.)

(Accepted for publication 19 December 1955)





1



2a



2b



2c,

Trans. Brit. mycol. Soc. **39** (4), 449-459 (1956).

GRIPHOSPHAERIA NIVALIS (SCHAFFNIT) MÜLLER & VON ARX AND LEPTOSPHAERIA AVENARIA WEBER ON OATS

BY MARY NOBLE AND ISABEL G. MONTGOMERIE*

Department of Agriculture for Scotland

(With Plate 19)

Oats in Scotland are attacked by *Griphosphaeria nivalis* which causes seedling blight and brown foot-rot; also by *Leptosphaeria avenaria* causing leaf-spotting and stem-breaking. Both diseases are transmitted through the seed. Perithecia of *Griphosphaeria nivalis* are formed freely on infected plants but those of *Leptosphaeria avenaria* have not been found in Scotland.

Surveys have recently been made in Scotland to determine the pathological condition of oat (*Avena sativa*) seed samples sent to the official Seed Testing Station, Edinburgh, for routine purity and germination tests. Field crops have also been examined in connexion with the Inspection Scheme for seed oats arranged by the Department of Agriculture for Scotland, and in the course of this work certain information on the occurrence in Scotland of *Griphosphaeria nivalis* and *Leptosphaeria avenaria* has accumulated. The field symptoms of the diseases which they cause have already been illustrated and briefly described by Noble & Montgomerie (1954).

Griphosphaeria nivalis

Historical

In the imperfect state, *Fusarium nivale*, this fungus has been known throughout the world as the 'snow-mould' of grasses since it was given the specific epithet '*nivalis*' by Fries (1825); partly because it attacked cereals and grasses when they were covered by snow and partly because of the abundant white mycelium. Later it was recognized on the Continent of Europe and elsewhere to cause pre-emergence blight and foot-rot of cereals. In Britain, although 'snow-mould' is well known on turf, it is not recorded in cereals, while cereal foot-rot and pre-emergence blight have generally been ascribed to other species of *Fusarium*. Bennett (1933), however, pointed out that *F. nivale* attacked cereals in the north of England. In Scotland, a survey made by Dennis (1943) showed that *F. nivale* was commonly seed-borne in oats, and in laboratory and plot experiments it proved to be a virulent pathogen. No field observations were made at that time.

The perithecial state was at first thought to be that collected by Berkeley and Broome in England in 1850, named by them *Nectria graminicola*

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(Berkeley & Broome, 1859), a fungus now known to be unconnected with *Fusarium nivale*. Wollenweber (1913) obtained perithecia in a pure culture of *F. nivale* derived from wheat from America and assigned them to *Calonectria* making the combination *C. graminicola* (Berk. & Br.) Wollenw., whereas Weese (1913) examining the perithecia on plants identified them as *Leptosphaeria* or *Metasphaeria*, and erroneously concluded that they could not therefore be the perfect stage of *F. nivale*. Schaffnit (1912) proposed *Calonectria nivalis* Schaffnit for a new taxon, and this name is in current use in Great Britain. Müller & von Arx (1955) have now proposed the combination *Griphosphaeria nivalis* (Schaffnit) Müller & von Arx, pointing out that Schaffnit and Wollenweber studied the fungus in culture, where the perithecia are formed superficially; in nature they are formed within the tissues, a point which they consider taxonomically important.

The recorded distribution of the perfect state of this fungus throughout the world does not correspond with that of the imperfect. In Britain the perithecia seem to have remained unrecorded, although Bennett found immature perithecia in his cultures and thereafter searched unsuccessfully for them in the field. During the present investigation they have been found to occur abundantly on plants in the field (Pl. 19, fig. 1) as well as in culture. The identity of these perithecia has been repeatedly checked by making single ascospore isolations and from them obtaining *Fusarium nivale*. In America, although 'snow-mould' is recorded on winter wheat (Blodgett, 1946), the perfect state has not been described except by Wollenweber; Oswald (1950) did not find the perithecia in California. Gordon (1952) states: 'Perithecia of *Calonectria nivalis* Schaffnit have not been recorded from nature in Canada.' Prof. Jamalainen (private communication) has found the perfect state in Finland rather rarely on the stalks of winter rye, but not on other cereals. From Russia Dobrozrakova (1928) reported that in the beginning of July pinkish and later light brown perithecia were found in the fields of wheat and rye, and Lind (1913) in Denmark also found them 'common on rotten grasses f. inst. *Secale cereale* in June'. Müller & von Arx (1955) describe their occurrence in Switzerland as frequent.

In the present survey of oat seed during two seasons 1951-3 it was found that the large majority of the samples examined had at least 10% infection by *Fusarium* spp. and that the species occurring most commonly was *F. nivale*. The provenance of the seed was not specially investigated but most of it was grown in Scotland. It is interesting to note that in Eire a commonly occurring *Fusarium* on cereals is *F. graminearum*, the imperfect state of *Gibberella zeae* (McKay & Loughnane, 1945), a species not found in the present survey although it does occasionally appear in Scotland. *F. culmorum* and *F. avenaceum*, for which no perfect states are known, were found fairly frequently, sometimes on the same seed as *F. nivale*.

The disease in spring-sown oats

The importance of seed-borne as against soil-borne infection was first pointed out in Germany by Hiltner & Ihssen (1911). Seed-borne infection was also found, as already mentioned, in Scotland in 1943, but Sprague

(1950) infers that in North America this does not occur and this is confirmed by Gordon (1952) from his survey of cereal seed in Canada.

Seed which is severely infected fails to emerge from field soil, especially from heavy soil, and 'pre-emergence blight' results. Depth of sowing affects the severity of the disease since the more attenuated the coleoptile, the more severe the attack by seed-borne and soil-borne fungi.

Less severely infected seedlings which may penetrate the soil are weakened, the roots and coleoptile being brown, and often the base of the first leaf is attacked so that the blade lies flat on the soil. Occasionally buff spots develop on the leaf. It is sometimes difficult to determine the presence of *F. nivale* on such plants without making isolations since unless the plants are collected during a period of damp weather the conidia are not easily found.

The perithecia begin to appear in oats in Scotland in late May when the plants are at the five- or six-leaf stage. The young fructifications are pink or buff and quite inconspicuous against the brown colour of the infected culm. They ripen and shed their spores quickly as the stem elongates, those on each leaf-sheath ripening more or less together and becoming darker in colour. Perithecia have so far only been found on the upper part of the sheathing leaf-bases, on the culm and extreme base of the infected leaves (Pl. 19, figs. 2, 3) not in the leaf blade. The recorded distribution of the perithecia in those countries where *F. nivale* is known to be present certainly suggests either that their formation depends upon some close correlation between the parasite and host, probably an effect of environment not yet understood; or that they are present but unrecognized. In Scotland they play an important part in the dissemination of the fungus. The asci are very thin-walled and the ascospores, which in shape and size are similar to the conidia, are readily shed. They germinate immediately so that they resemble conidia functionally as well as morphologically. The perfect state is, therefore, not primarily an overwintering form; transmission in spring-sown oats takes place effectively through seed-borne infection.

Schaffnit showed that the degree of infection of the seed depends upon the stage of its development when infection takes place. Thus, infection occurring between anthesis and the beginning of ripening becomes deep-seated, and this he termed 'primary infection', but infection occurring later remains superficial or 'secondary'. Seed with primary infection is shrivelled and useless for sowing as the seedlings, if any, which arise fail to penetrate the soil and so bring about 'pre-emergence blight'. These classes actually overlap and the response to seed treatment depends on the depth of penetration of the fungus.

Determination of infection in seeds

Three methods of testing seeds for infection by *Fusarium* spp. have been used in this survey:

(1) Seeds were treated with a mild disinfectant, plated on agar, and the fungi obtained in pure culture for identification.

This method had the advantage that the species of *Fusarium* involved can usually be determined at once even without subculturing. The medium

used was potato-dextrose agar; the seed was disinfected in 5 % Chlorox (a fixed sodium chlorite preparation), plated without subsequent washing, and incubated for about 10 days at approximately 14° C. Infection is then determined according to the fungal colonies arising from the seeds, but care is required to identify correctly the fungi involved. Thus the worker inexperienced in the genus *Fusarium* is apt, when examining the fungal colonies, to overlook the white or pale pink mycelium of *F. nivale* and to select as belonging to the genus, only the strikingly red mycelium of *F. culmorum* and *F. avenaceum*. Even the saprophytic *Epicoccum* sp., which is common on oat seed in Scotland, can be confusing in culture because of its sterile orange-red mycelium. Furthermore, spore production is often delayed in *F. nivale* which, however, has one useful distinguishing feature in that the mycelium is usually stranded in appearance even in very young colonies. The disadvantages of this method are that determination of the fungi must be carried out by a mycologist, also that deep-seated and superficial infection are not distinguishable from each other.

(2) Seeds were incubated in a damp atmosphere so that primary roots were formed; infection being determined by brown discoloration of these roots.

Incubation in a damp atmosphere was described and illustrated by Doyer (1938) who used the method in making routine determinations of *Fusarium* spp., including *F. nivale*. For this purpose the seeds are incubated in metal trays or Petri dishes lined with damp filter-paper, or in any similar humidity chamber at room temperature. Unless infection is very deep-seated, the seeds germinate and the primary roots are formed in about a week. The presence of *Fusarium* spp. is determined by the appearance of brown discoloration at the base of the primary roots, sometimes extending on to the base of the coleoptile (Doyer, 1938, pl. IV). Conidia diagnostic of the species are not usually present on these lesions, and it has not been found possible to differentiate between the lesions caused by the different species. Isolations can, however, easily be made from the infected roots.

(3) An adaptation of the 'Hiltner' test was used in which the seedlings are planted in a substrate which aggravates the disease so that diseased seedlings are easily distinguishable.

Hiltner, whose name is associated with this method, was a pupil of Nobbe and director of the Munich Station of Agricultural Botany, later the Station for Plant Protection of which the seed-testing station was a subsection. Hiltner gave Ihssen the task of investigating the 'snow-mould' of cereals and, when seed-borne infection was thereby shown to be important, they devised a method of testing seed for its presence (Hiltner & Ihssen, 1911). The method as originally devised was to plant seeds under a layer of ground brickstone which, because of its capillarity, had high water-holding capacity and was fairly heavy. The layer was about 3 cm. deep so that the seedlings had to penetrate a considerable mechanical barrier in high humidity similar to adverse field conditions. In the present investigation 'vermiculite' was used in place of brickstone as it also has very high water-holding capacity and is much more convenient to handle and sterilize. In each test two replicates of fifty seeds are planted under 3.75 cm. (1.5 in.) of vermiculite in cardboard boxes 17 × 5 cm. and 8 cm. deep, and

incubated in a glasshouse for 14 days at 12–15° C. The seedlings then have formed primary roots and the coronal roots are beginning to appear; the plumule is green and about 5 cm. long. The vermiculite is easily washed off and the seedlings examined for symptoms of foot-rot. Healthy seedlings can penetrate both brickstone and vermiculite; those infected superficially may also emerge but the coleoptile is discoloured. The discoloration caused by *Fusarium* spp. is brown and extends all round the coleoptile and can usually be distinguished from that caused by *Helminthosporium avenae* which takes the form of a streak. Frequently, however, seedlings are infected by both fungi.

Severely infected seedlings become twisted and remain below the surface of the medium ('pre-emergence blight'). Some which would fail to emerge from brickstone can penetrate vermiculite but these bear the characteristic discoloration of the coleoptile which enables a determination to be made whether *Fusarium* or *Helminthosporium* is present. Another advantage of the vermiculite test is that in oats leaf-spotting often occurs on the emerged plumules and this also helps diagnosis. *Fusarium nivale* can cause rather water-soaked buff or reddish spots but they are usually confined to the leaf base, whereas *Helminthosporium* spots are pale with dark reddish purple margin and run up the leaf usually in a straight line. The leaf-spots caused by halo blight (*Pseudomonas coronafaciens*) are smaller, usually reddish, water-soaked and scattered.

Germination capacity of infected seed

Testing seed for germination, in so far as field planting value is concerned, is governed by the Rules of the International Seed Testing Association. It has been shown that infection by *Fusarium nivale* is transmitted from the seed to the seedling, and that according to the severity of the infection and the conditions under which the seed germinates the seedling may be attacked to a greater or less degree. Under the current International Rules for Seed Testing (I.S.T.A., 1953) 'it is not correct to regard the total number of sprouted seeds as representing the germination of the sample, but on the contrary, only the seeds producing normally developed and healthy seedlings'. It is understood that in this context 'healthy' does not necessarily infer complete freedom from disease and is therefore a point requiring careful consideration when dealing with oat seed infected with *F. nivale*. If the test is made on blotters the primary roots of infected seedlings will show the discoloration described above but will otherwise appear normal and are indeed morphologically normal. Another substrate used in routine testing of oat seed is damp sand on which the seed may be laid or very lightly pressed in. Under these conditions the mycelium of *F. nivale* develops freely forming to some extent 'snow-mould' which not only attacks the infected seedlings from which it arises, but also adjacent healthy seedlings. The primary roots of infected seedlings become brown and rather shrivelled and apparently devoid of root-hairs. The latter are, however, present but closely adpressed to the root by the fungal mycelium. A third accepted method is to plant the seeds very lightly in sterilized compost with the embryo end down and the distal end projecting. The roots grow

into the substrate and symptoms of attack by *Fusarium* are minimized; while the coleoptile under such favourable conditions grows rapidly although it may become brown in infected seedlings. In using these tests the analyst must decide whether the seedlings are normal and are such as could be 'expected to produce plants under favourable conditions' or are abnormal and therefore of 'no practical planting value'.

Schaffnit distinguished (a) *Keimfähigkeit*—germination capacity, (b) *Keim-schnelligkeit*—germination energy, and (c) *Triebkraft*—shooting vigour. The germination test according to the International Rules measures the germination capacity, whereas the Hiltner test, still used in Sweden for all cereal samples, reveals the 'frequency of such diseases as *Fusarium*' (E.P.P.O., 1954) which can cause reduced shooting vigour.

Control

Control of this disease is closely bound up with the problem of the relative importance of the soil or seed as sources of infection. Emphasis is generally laid upon the importance of the soil in this respect, but the results of the surveys of seed oats in Scotland strongly suggest that the seed is equally important and indeed the difficulty of finding seed free from infection at present precludes experiments to assess the importance of soil-borne infection. Disinfection with organo-mercurial dusts undoubtedly controls a very high proportion of seed-borne infection, especially if it is superficial but, as has been shown, some may be deep-seated and therefore unamenable to treatment by organo-mercurial dusts at present available in this country.

It is sometimes stated that brown foot-rot is only severe in acid soils and that liming would therefore control the disease, but this is not the case if *F. nivale* is the causal species. In the present investigation severe infection occurred in a crop on soil of pH 7 and another of pH 6.1, while Schaffnit & Meyer-Hermann (1930) described *F. nivale* as favoured by alkaline soil. A more important factor is probably the quick establishment of the plant. A finely worked seed-bed, fairly shallow sowing and the use of treated seed of high germination energy and 'triebkraft' are recommended as control measures.

Leptosphaeria avenaria

The first British record of this fungus, of which the imperfect state has been known as *Septoria avenae* appears to be by Trail (1887) when it was collected and described as *S. affinis* causing a leaf-spot of oats, but no herbarium material is available to support this record. Frank (1895) later described it in Germany as *S. avenae* and Weber (1922), in America, gave a full account of the disease (then of negligible economic importance) and of the causal fungus including the first record of the perfect state which was formed in culture. In 1948 *S. avenae* was found in Scotland and Wales, causing not only leaf-spotting but straw-breaking, and in the same year it was noted in America by Meehan & Murphy (1949), while in 1952 'an epidemic outbreak reduced yield and quality in many oat-growing sections in Iowa, the Dakotas, Minnesota and Wisconsin' (Miller, 1952), and it has been found

in parts of Eastern Canada causing loss of crop (Dep. Agric. Canada, 1952). The perfect state was first recorded in the field by Meehan & Murphy in America as 'occasionally present', whereas Huffman (1955) reports perithecia to be 'formed freely on overwintered oat stubble in early summer'. It has not yet been found in the field in Britain.*

Studies by Poole & Murphy (1953), Simons & Murphy (1952) and Huffman comprise a very full account of the course of the disease in America, while Johnston (1952) described the fungus in cultures. In the present investigation micro-pycnidia have been found on leaves in the field before harvest, their early occurrence suggesting that the disease may take a different course in Scotland from that described in America. Seed-borne infection is also believed to occur in this country but not in America.

The disease in the field

In Scotland the first symptom of *Septoria* infection takes the form of fine black necrotic streaks on the base of the coleoptile (Pl. 19, fig. 4). This has been found in seedlings at the two-leaf stage as well as in seedlings grown in sterile vermiculite or sand. Unless the seedlings are washed carefully before they are examined these lines are easily overlooked; *Septoria* has been isolated from them, although with difficulty. In America much more severe symptoms in the seedlings grown from naturally infected seeds have recently been described by Lund & Shands (1955), but these authors do not consider that the mature crop becomes infected from such inoculum. Certainly in Scotland, as in America, there is a period after the seedling stage during which no sign of this disease has been found, but it remains to be proved whether later infection cannot arise from inoculum persisting in the soil from previous crops or from inoculum introduced with the seed. In America seedlings from severely infected seeds are described as failing to emerge or, on the other hand, recovering from the disease.

The first obvious signs of infection in the field usually occur in early July when the plants are almost full-grown. A dark purple-brown leaf-spot with orange margin appears on any part of the leaf-blade. This is very easily confused with the leaf-spot caused by *Helminthosporium avenae* but whereas the *Septoria* spot is lozenge- or diamond-shaped, the former is usually linear and much lighter in colour. Sometimes the two diseases are very closely associated and may even form one confluent lesion. In such cases if the lesion is examined carefully with a $\times 12$ hand lens under reflected light, the conidiophores and conidia of *Helminthosporium* can be identified, while by transmitted light the pycnidia of *Septoria* appear as translucent pin-points. The spores, which mature quickly, are pink *en masse* and do not readily form tendrils, but seem to be easily spread in water from the point of origin. The disease thus appears at the base of the leaf where the blade passes into the sheath, and as Huffman also remarks it seems likely that spores germinate in the moisture held by the upstanding ligule. As the internode lengthens this lesion becomes dark grey-brown, contains pycnidia

* One of us (M. N.) has now, June 1956, found perithecia believed to be those of *Leptosphaeria avenaria* on year-old stubble.

and occupies an internodal position. The underlying culm becomes dark brown or very dark grey but no pycnidia have been found in these underlying tissues. The work of Poole & Murphy indicates that such discoloration is caused by the toxin which the fungus produces, particularly when dextrose is available. Culms which have thus become diseased appear to be more brittle than healthy straw and certainly they tend to break at the point of infection especially if 'ripening' is slow. The culm lesion often takes the form of a zoned band and infection also is found at the nodes on which pycnidia and later micropycnidia are freely formed. The rachis, glumes and kernels may be affected by the disease, and severely infected seed is characteristically discoloured with pycnidia present in the pales, but in Scotland seed without such discoloration may also give rise to infected seedlings.

By late July or August micropycnidia with minute bacterium-like spores can be found in the withered basal leaves and also in the outer glumes. They are not readily detectable in fresh material but, like the macropycnidia, become more apparent if treated with hot lactophenol and cotton blue. Micropycnidia with spores have also been found, each situated on top of an empty macropycnidium forming a double fructification. Both macropycnidia and micropycnidia tend to form below the stomata and the osteole often coincides with the stoma-pore. The wall of the pycnidium is sometimes black but in others is transparent, and this does not seem to be a question of thickening with age. The pycnidia are formed abundantly on the leaf-sheaths, particularly those of the upper leaves, and are not unlike the perithecia of *Griphosphaeria nivalis*, but the latter become more conspicuous as they become opaque with age, whereas the pycnidia are translucent. The spores exude as a pink globule. The perfect state was distinguished by Weber from *Leptosphaeria avenae* Auerswald, which it otherwise closely resembles, on account of the latter having hyaline spores $3.5-4 \times 15-16\mu$, measurements which are remarkably close to those of the hyaline 3-septate ascospores of *Griphosphaeria nivalis*.

The stem base of the mature infected plant often bears a black necrotic lesion running up between the prop roots, and the fungus has been recovered in pure culture from such lesions. It is not known what effect, if any, such lesions have on the functional or structural efficiency of the stem, but it is possible that the blackening is caused by the toxin which the American workers have demonstrated, and that this may cause brittleness of the straw. It has been observed in Scotland that *Septoria avenae* is most severe in its effect in tall well-grown crops which are in any case most liable to lodge. The infected straw may be reduced in feeding value and American work suggests that infection may be spread by using manure made with such straw. No evidence that this takes place in Scottish crops has so far been obtained, whereas it has been shown that infection can be seed-borne.

Detection of infection in seed

The detection of infected seed other than those bearing discoloration and fructification presents certain difficulties. The usual laboratory method of plating on agar has proved unsuccessful in the present investigation owing

to the very frequent occurrence of *Helminthosporium avenae* and *Fusarium* spp. (especially *F. nivale*) which are much quicker growing and may occur on the same seed as *Septoria*. Experiments are at present proceeding in order to devise a differential medium which may suppress these other fungi and favour the growth of *Septoria*. The 'damp chamber' method also has failed to reveal infection.

Planting seed in sterile sand or vermiculite as in the modified Hiltner test described above has been more successful. Symptoms of *Septoria* infection have been determined as one or more sharp black necrotic streaks on the coleoptile, sometimes in a series parallel to one another, and about 2 or 3 mm. in length. They may occur on any part of the coleoptile but more often on the basal part below the coronal roots and may even be under the glumes (Pl. 19, fig. 4). *Helminthosporium* and *Fusarium* may also be present and, by dissecting out the affected parts, all three fungi have more than once been isolated from one seedling. In order to confirm that *Septoria avenae* was the cause of the sharp black necrotic streaks on the seedlings attempts were made to inoculate seeds with pure cultures.

Inoculum was prepared by flooding the surface of oat agar with a suspension of macroconidia and hyphae in sterilized water and incubating at about 20° C. for 1 month. Numerous pycnidia had then formed and the macroconidia were emerging as pink/brick red globules. A spore suspension of macroconidia was prepared by flooding the surface of the agar with sterilized water and decanting. The same stocks of two varieties of oats, Early Miller and Victory were used as host plants.

In one experiment, glumes were removed from about 200 seeds. One hundred were then placed overnight in a Petri dish containing a macroconidial suspension of *S. avenae*, the remainder in sterilized water and then planted in vermiculite as for an infection test. After germination 69 % of the inoculated seeds had produced seedlings showing symptoms of *Septoria* infection. No symptoms developed on the controls. *S. avenae* was recovered in pure culture from a number of the inoculated seedlings.

In another experiment a suspension of microspores was used. Seeds were soaked overnight in the suspension or in sterile water. One hundred seeds from each treatment were sown in sterilized Vermiculite, and after 14 days the resulting seedlings were examined for symptoms. The results of this experiment were as follows: Early Miller, inoculated—20 seedlings infected, controls healthy; Victory, inoculated—14 infected, control 1 infected. Isolations were made from all 34 seedlings showing symptoms of *Septoria* and the pathogen was recovered in pure culture from 10, only saprophytic fungi were obtained from the remaining 24.

As it was assumed that seed infection in the field would take place sometime between anthesis and seed maturity an attempt was made to bring about infection at this time under artificial conditions. Early Miller plants were therefore grown in the glasshouse and inoculated at frequent intervals by spraying with a suspension of macro-spores and directing the inoculum at open glumes. After each inoculation the plants were kept in a humid atmosphere for 48 hr. Controls were similarly treated but were sprayed with tap water instead of a spore suspension. Symptoms of infection similar to those found in the field were obtained on inoculated plants

while none occurred on the controls. After harvesting in August, seeds were sown in November. The percentage germination was very low but 9% of the resulting seedlings showed symptoms of infection with *Septoria*. These experiments nevertheless confirm that the 'black streak' symptom on seedlings is caused by *S. avenae*. The cycle of the disease in America has recently been described by Huffman (1955). Seed-borne infection in Iowa is not significant, if it occurs at all, but micropycnidia and perithecia forming on overwintered stubble in early summer provide inoculum for the leaf-spotting phase. In Scotland micropycnidia have been found as early as August in old leaves and in November on stubble, but the perithecia have not yet been identified although those of another species of *Leptosphaeria* have been found. On the other hand, the necrotic streak type of seedling infection has not been described from America. Further investigation of *Leptosphaeria* spp. in this country may show that the perithecia are present but overlooked or wrongly identified as has apparently been the case with *Griphosphaeria nivalis*.

G. nivalis and *Leptosphaeria avenaria* have proved to be linked with one another in pathogenicity as well as mycologically. They can often be found on the same plant, even within one seed; and in each the imperfect state has long been known as a plant pathogen, whereas the perfect state has been relatively little known. The confusion concerning the identity of the perithecia of *Griphosphaeria nivalis* had undoubtedly had repercussions on the study of the etiology of Brown Foot Rot of cereals in Great Britain and perhaps elsewhere. Thus Weese (1913), having examined and identified a perithecial fungus as a *Leptosphaeria* or *Metasphaeria*, but neither *Nectria* nor *Calonectria*, wrongly concluded that it could not therefore be the perfect state of *Fusarium nivale*.

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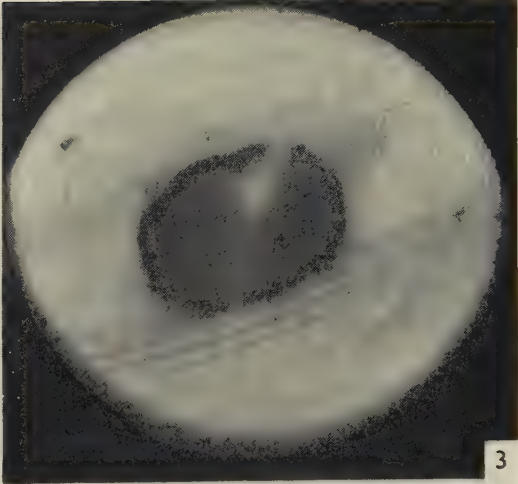
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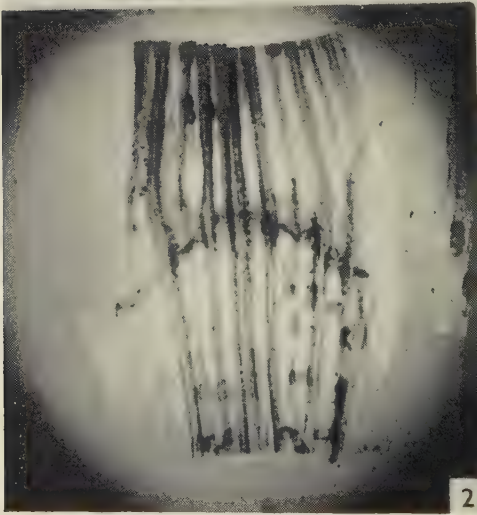




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EXPLANATION OF PLATE 19

Griphosphaeria nivalis

- Fig. 1. Perithecia on culm of oat plant ($\times 3$).
 Fig. 2. Perithecia on young leaf sheath of oat plant ($\times 12$).
 Fig. 3. Perithecium ($\times 145$).

Leptosphaeria avenaria

- Fig. 4. Oat seedling showing symptom of infection ($\times 4$).

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THE CONIDIAL APPARATUS OF *TRICHOTHECIUM ROSEUM*

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(With 2 Text-figures)

Trichothecium roseum is redescribed. Spores are produced in basipetal succession from the tip of the conidiophore. The first-formed spore is morphologically distinct from those formed later. The nature of the macroconidia (whether phialospores or terminal thallospores) is discussed.

Although *Trichothecium roseum* Link ex Fries (= *Cephalothecium roseum* Corda) is such a common and well-known mould, the morphology of the conidial apparatus does not seem to have been clearly understood. Of the early mycologists who studied the fungus, Link (1809) did not show the spores attached, and Corda (1838) figured the spores in a head at the end of the simple conidiophore apparently directly attached to its apex. Amongst recent workers Smith (1954), who gives an excellent photograph of the spore chains, remarks that the sporophores are erect 'bearing terminal clusters of spores directly attached to the tip'. Barnett (1955), in his laboratory handbook of conidial fungi, gives a curious figure of the fungus which seems to indicate that the spores are associated with slime at the end of the conidiophore. They are, in fact, dry spores readily detached by air currents. Matruchot (1892), who made a careful study of the fungus and demonstrated the production of slimy, unicellular microconidia (phialospores) under certain cultural conditions, appreciated that macroconidia are produced in succession from the end of the sporophore, and as a result a chain is formed in which the spores are packed together rather like the spikelets in the ear of wheat. However, his figures show the individual spores linked together so that the lower cell of each spore is in open communication with the lower cell of its two neighbouring spores in the chain. Elliott (1920) gave an account which is in very close agreement with that of Matruchot, although she was apparently unaware of his work. She says 'each conidium including the first arises obliquely sometimes even horizontally at the apex of the conidiophore, this is cut off from the conidiophore by a cell wall, and beneath the cell wall the hypha swells out into another conidium also obliquely placed and opposite the other one, but still attached to it and in its turn, it becomes cut off and so the process continues, conidia one after another in a close spiral being produced by the swelling out of the apex of the conidiophore; and thus a chain is produced'. This is quite correct except for the statement that the first conidium arises obliquely. Unfortunately her figures are rather misleading and, like those of Matruchot, show the spores of the chain in open communication.

Because of the confusion and inaccuracies in the published accounts of the morphology of this fungus and because conidial development is likely to become more and more important in the classification of Hyphomycetes (see Hughes, 1953), it is felt that a redescription is desirable.

The conidiophore is usually simple, erect, 100–250 μ long and 2- to 3-septate with the cross-walls limited to the lower half. When young it tapers gradually but slightly towards the apex and its surface is roughened by minute granules. The first spore is cut off terminally, its longitudinal axis being in line with that of the conidiophore (Figs. 1 A, 2 A). This feature, clearly illustrated by Matruchot (1892), was missed by Elliott (1920). Mason (1933) remarks: 'It is not easily displaced, and under moist conditions further growth at the apex of the sporophore produces the characteristic "ear" of spores.' This seems to suggest that the spore remains attached to the apex of the conidiophore, which is not so. The terminal spore normally is almost fully grown before it is delimited from the conidiophore by a cross-wall. The wall dividing the spore into two cells may not be formed until some time later. After the first spore is formed the apex of the conidiophore immediately below blows out into a minute swelling only slightly wider than the end of the conidiophore. The wall of this is not uniformly thickened. Where it is in contact with the first-formed spore the wall is thicker and this thickening persists when the swelling enlarges into the mature spore (Fig. 2 C, D). This second spore expands laterally so that its axis is at an angle of about 110° to the conidiophore (Figs. 1 C, 2 D). When the spore is fully or almost fully grown, it is cut off by a cross-wall. The third spore arises below the second in the same way, but this time it swells out on the other side. So a chain is produced in which each spore, except for the first one, is more or less horizontal, and alternate spores point to left and right (Fig. 1 E, F). This two-ranked chain, which may finally contain 20–30 spores, normally becomes spirally twisted as it increases in length, and may topple over to some extent under its own weight.

When liberated spores are critically examined, it is easy to distinguish between first-formed spores and others. The two-celled spore has a constricted base. In a first-formed spore this is relatively narrow and the detachment scar, which is outlined by a very slightly raised rim of wall material, is symmetrically placed at the base (Fig. 2 B). In later-formed spores (Fig. 2 C) the basal constriction is wider and the scar is to one side of the base. In the corresponding position to the other side of the base a slightly thickened region of the conidium wall, representing the position of contact with the next spore above in the chain, can usually be recognized.

As already noted the conidiophore is at first a straight and slightly tapering hypha, but, as more and more spores are abstricted, it tends gradually to widen distally so that the older conidiophores are often quite cylindrical.

In producing a basipetal chain of spores without any change in length, the conidiophore resembles a phialide, but its shape is not right. There is no basal swelling and no distal neck. There is no trace of an apical collarette. In my own experience with aquatic Hyphomycetes, terminal thallospores (aleuriospores) are delimited from the conidiophore at an early stage in development, whilst phialospores are not cut off until they are fully

mature. The macroconidia of *T. roseum* agree with phialospores in this characteristic, a delimiting wall being formed only when the spore has almost reached its full size.

Hughes (1953) has described a number of examples of conidiophores (e.g. *Scopulariopsis*) which look like phialides, but in which the tip of the

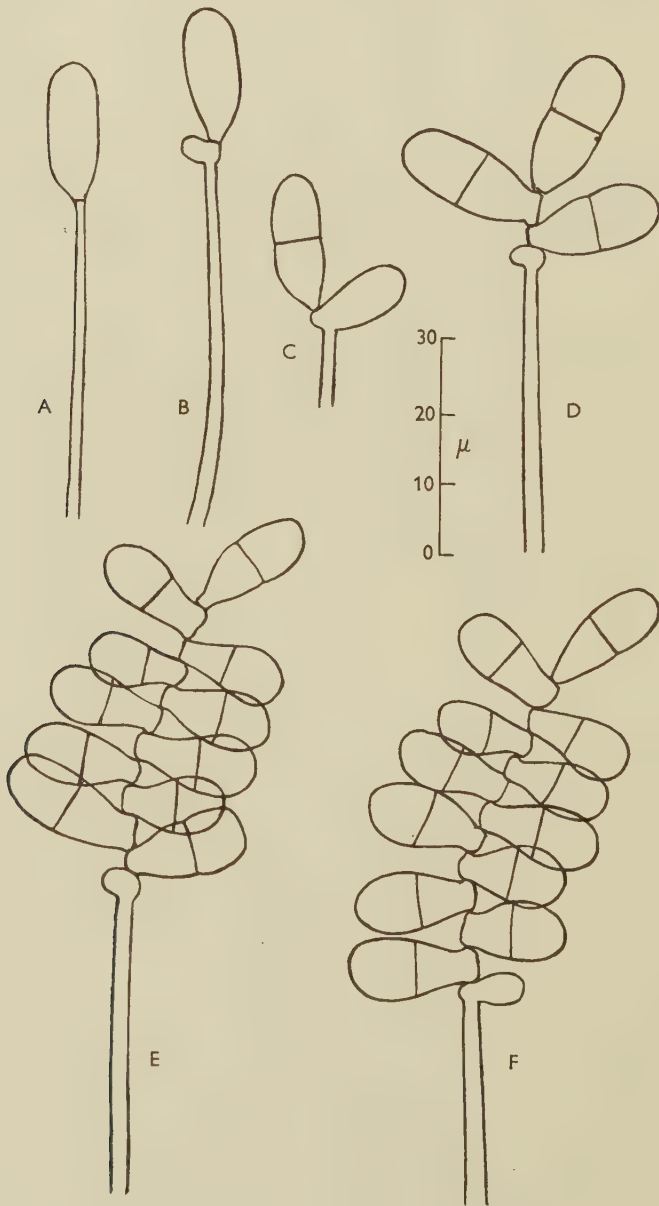


Fig. 1. *Trichothecium roseum*. A-F, conidiophores of increasing age.

conidiophore gradually elongates and is characterized by ring-like markings. In this type, to quote Hughes, 'a plurality of conidia develops from a single conidiophore through the conidial scar left by the previous conidium; by a repetition of this proliferation a succession of conidia is produced accompanied by successive increase in the length of the conidiophore'.

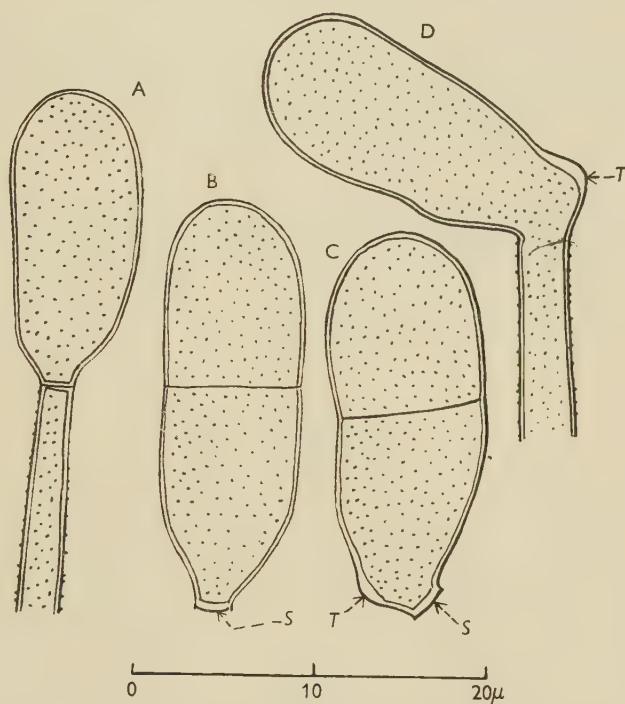


Fig. 2. *Trichothecium roseum*. A, end of conidiophore with a first-formed spore; B, a liberated first-formed spore; C, a liberated later-formed spore; D, nearly full-grown later-formed spore (the spore above it is not shown). S, detachment scar; T, region of thickening in zone of contact with a sister spore of a chain.

It seems to me that the conidiophore of *Trichothecium* may represent a modification of this type where the level at which each successive spore is cut off remains exactly the same, so that no elongation of the conidiophore and, therefore, no annular scars are produced.

Although I am inclined to regard these spores as terminal thallospores (aleuriospores; see Mason, 1933), it is difficult to say exactly why they should not be classified as phialospores except, perhaps, that the conidiophore does not look like a conventional phialide and that the species already appears to have in its microconidia a typical phialospore stage. This does, however, raise the question of whether there is, in fact, any fundamental difference between phialospores and aleuriospores.

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THE ISOLATION OF DERMATOPHYTES FROM CLINICAL MATERIALS

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The diagnosis of infection and the isolation of dermatophytes from clinical materials are briefly discussed, and the results of a series of isolations by a strictly defined method are given. The results are analysed and evidence is presented which indicates that specimens infected with *Trichophyton mentagrophytes* (all but two isolates were of the *interdigitale* form) differ, either in quantity or distribution of hyphae, from those infected with *T. rubrum*. The treatment of heavily contaminated material with fungicides is suggested as a means of reducing the growth of contaminating moulds and bacteria and increasing the isolation rate.

INTRODUCTION

Diagnosis of infection by dermatophytes may be made in the laboratory, either by recognition of the hyphae of these fungi in the tissue by microscopy, or by isolation of the fungi in pure culture.

Microscopic examination is usually carried out by clearing the specimen in 10-20 % KOH, but material may be stained if desired by the Periodic acid-Schiff method (Kligman, Mescon & de Lamater, 1951), with Carbol-Gentian violet (Adamson & Annan, 1949), or Chlorazol black (Curry 1949). Microscopy of infected hair may give some indication of the species of dermatophyte present, though culture is still necessary for specific identification. No such indication is given by examination of skin or nail specimens. On the other hand, it is our experience that the hyphae of dermatophytes are readily distinguishable from the hyphae of non-pathogenic fungi occasionally present in clinical specimens, and we believe the suggestion put forward by Sagher (1954), that the presence of such fungi might lead to wrong diagnoses is unlikely to be true.

Many different media can be used for isolation of ringworm fungi; those given by Riddell (1951) as in most general use are Sabouraud's dextrose agar and 4 % malt-extract agar. For certain species with low growth rates however, e.g. *Trichophyton verrucosum*, enriched media such as nutrient agar (Austwick, 1954) or Sabouraud's dextrose medium supplemented with thiamin and inositol (Georg, 1950) are probably preferable. In order to reduce the growth of bacterial contaminants, potassium tellurite or a penicillin-streptomycin mixture may be incorporated in the medium. Cleaning of the site with spirit before the specimen is taken, and allowing the material to dry before inoculation, also reduces the growth of these contaminants. In addition, specimens known to be heavily contaminated may be immersed in 70 % alcohol for about 1 min. immediately before inoculation.

The development of saprophytic moulds is retarded for a period if cycloheximide (Actidione)* is incorporated in the medium (Georg, 1953). As will be shown later, contamination by both bacteria and moulds will be reduced if the specimens are treated with fungicidal preparations before inoculation to the media.

I. SKIN AND NAIL SPECIMENS

So far as we are aware, the results of a large series of isolations by a strictly defined method have not previously been published; for that reason alone, if for no other, the following account would seem to be of interest.

Materials and Methods

The material was taken by Dr J. G. Holmes during a survey of foot ringworm in coal miners in Great Britain (Gentles & Holmes, 1955). The specimens were sent to the laboratory by post, each enclosed in a folded slip of paper (approx. 5×5 in.). In the laboratory standard treatment was given to each specimen as follows.

The paper was unfolded and kept flat by placing a block of oak wood $5 \times 1 \times \frac{1}{2}$ in. at each end, and the specimen of skin or nail was divided into fragments each of about 1 mm. in diameter, by means of a mounted needle and a no. 15 Swan Morton scalpel. The scalpel and needle were flamed between specimens.

Culture. Twelve fragments of each specimen were inoculated for culture, four to each of three slopes of agar containing one or other of the media as described below. At first Sabouraud's dextrose agar, 4% malt-extract† agar, beerwort agar and all of these media with penicillin (20 units/ml.) and streptomycin (40 units/ml.), or with potassium tellurite (0.036%, w/v) were used in various combinations. The most successful proved to be malt-extract agar containing potassium tellurite, and two tubes of this medium and one tube of plain malt-extract agar were selected for use during the survey proper. (This combination was that used by one of us (J. C. G.) during a period of instruction under Dr Jacqueline Walker at the London School of Hygiene and Tropical Medicine in 1951.) Although the selection of a medium for use in isolation is often largely a matter of personal inclination, we think that 4% malt extract, because of its low unadjusted pH and high vitamin content, is perhaps preferable to Sabouraud's dextrose. Sanderson & Sloper (1953) reported that growth on beerwort and maltose-peptone agar was slow when tellurite was incorporated, but this was not our experience. Colonies appeared rather earlier on the 4% malt-extract agar medium supplemented with tellurite than on any medium without it, probably because the tellurite reduced even further the small number of bacterial contaminants encountered. We found no necessity to subculture dermatophytes from the tellurite medium, as advocated by Riddell (1951); normal colonies developed and remained viable for at least 6 weeks, and there was the added advantage that

* Actidione is obtainable from the Upjohn Co., Kalamazoo, Michigan.

† Extract of Malt manufactured by Boots Pure Drug Co. Ltd.

pigmentation by *T. rubrum* was produced more rapidly and was more intense on this medium than on the others.

Bacterial contamination was not particularly troublesome and the use of penicillin-streptomycin mixture, a more efficient antibacterial agent than tellurite, was not considered necessary. This was probably because the clinician cleaned the site thoroughly with surgical spirit before the scraping was taken; because of drying of the specimen (in this case, during transit) before inoculation; and because of the low pH of the medium. The amount of mould contamination was slight which is generally the case for human material other than nail.

Microscopy. Microscopy of four to six fragments (the quantity remaining in the majority of divided specimens after removal of inocula for culture) was carried out after clearing in 20 % KOH. As a check of efficiency, approximately 100 large specimens were selected for microscopic examination after staining by the periodic acid-Schiff method (Kligman *et al.* 1951), and also by clearing in KOH. Staining did not result in any increase in the number of positive diagnoses.

The results are summarized in Table 1.

Table 1.* *Summary of laboratory results in a survey of epidermophytosis*

No. of subjects examined	Positive for dermatophytes		Positive by micro- scopy and culture		Positive by microscopy only		Positive by culture only	
	% of no. examined		% of total positive		% of total positive		% of total positive	
	Total	% of no. examined	Total	% of total positive	Total	% of total positive	Total	% of total positive
2296	494	21	385	78	41	8.3	68	13.7

* The figures in Table 1 refer to the number of men examined. In all other tables they refer to specimens. At least two, and occasionally more, specimens were taken from each man.

Analysis of results

Microscopic examinations were noted only as positive or negative. For culture, however, which was carried out at 28° C. for a period of 3 weeks, records were kept of the number of colonies of dermatophytes obtained from the twelve inocula, and of the degree and nature of contamination.

(1) *Negative Culture*

We found more cases positive by culture alone than by microscopy alone, but nevertheless failed to culture 10 % of microscopic positives. Other workers (La Touche, 1954) report similar results. The reason for this failure is often not clear, but it is generally believed to be due to either overgrowth by saprophytic contaminants or inhibition of the dermatophytes by fungicides used in treatment. From experience of a series of cases under treatment, and from the few cases encountered when contamination was excessive, we have formed the opinion that previous application of fungicidal ointments or powders was probably the reason for failure to isolate in the majority of our cases.

(2) *Negative microscopy**

Almost 14 % of the positive cases were diagnosed by culture of a dermatophyte but not by microscopy (Table 1). The obvious explanation of failure to note the fungus on direct examination is that little of it is present, and it is probable that in such cases only a few of the possible twelve colonies of a dermatophyte will be obtained in culture. The results of microscopy of the specimens given in Table 2 suggest that this is true. The microscopic examination was only 50 % accurate when 1 colony of a dermatophyte was isolated but became more and more accurate as the number of colonies increased.

Several reasons can be suggested to account for the presence of only a small quantity of fungus in a specimen. Much of the specimen was not from an infected area; the fungus was of low virulence and invasion of tissue was slight; or vigorous host reaction prevented growth and spread of the fungus.

If either of the latter two suggestions is true such a condition might be a feature of one particular species. The two species most often isolated were *T. rubrum* and *T. mentagrophytes*, and details of microscopy and the number of colonies obtained from specimens infected with them are given for comparison in Tables 2 and 3. There is no significant difference between the two species with regard to the proportion of specimens which gave a low number (four or less) of colonies; the figures being for *T. rubrum* 55 % and for *T. mentagrophytes* 46 % (Table 3). On the other hand, the microscopy of such specimens infected with *T. rubrum* is apparently more accurate, only 21 % of them being missed, while 45 % of the same group of specimens were not diagnosed when *T. mentagrophytes* was the species involved (Table 2). *T. mentagrophytes*, because of its relatively higher growth rate, is probably more easily isolated in culture than *T. rubrum*. A possible explanation, therefore, is that when a limited amount of fungus which is liable to be overlooked on microscopy is present in a specimen, a culture is more likely to be obtained if the infecting species is *T. mentagrophytes*, than if it is *T. rubrum*. If this is true then some cases of infection with *T. rubrum* were possibly not diagnosed. In this connexion it should be noted that we carefully re-examined surplus material from men who had suspicious lesions but who were not proved to be infected; out of about 100 such cases only one extra positive case was found. There was unfortunately no material available for repeat culture in this instance and the species of dermatophyte which was present is not known.

Another possibility is that the findings are related to the different clinical symptoms usually produced by the two species. That material from the mild spreading infection of *T. rubrum* contains fungal hyphae in a reasonable and constant amount, while in material from the severe localized infection, often produced by *T. mentagrophytes*, the vigorous host reaction has reduced fungal development and the hyphae are present in a very small amount.

* This section is possibly applicable only to survey workers or to mycologists working apart from clinics. When the patient is available the usual procedure is to make a thorough examination by microscopy to confirm clinical diagnosis before setting up cultures.

Table 2. Correlation of microscopical results with the number of dermatophyte colonies grown from twelve inocula

	No. of colonies grown from twelve inocula						Total no. of specimens	
	1	2	3	4	4 or less	5 or more		
All species	No. of specimens No. not diagnosed by microscopy	97 49 (50 %)	90 29 (33 %)	67 18 (27 %)	69 10 (14 %)	323 106 (33 %)	326 23 (7 %)	649* 129 (20 %)
<i>T. mentagrophytes</i> only	No. of specimens No. not diagnosed by microscopy	59 35 (59 %)	47 22 (46·8 %)	29 8 (27·6 %)	28 8 (28·5 %)	163 73 (45 %)	192 16 (8·3 %)	355 89 (25 %)
<i>T. rubrum</i> only	No. of specimens No. not diagnosed by microscopy	36 14 (39 %)	40 7 (17·5 %)	38 10 (26·3 %)	41 2 (5 %)	155 33 (21 %)	127 7 (5·5 %)	282 40 (14 %)

* There were twelve specimens infected with *E. floccosum*.Table 3. The number of colonies of Trichophyton mentagrophytes and *T. rubrum* grown from twelve inocula

No. of colonies	<i>T. mentagrophytes</i>			<i>T. rubrum</i>			<i>T. mentagrophytes</i>			<i>T. rubrum</i>		
	Specimens	% of total	No. of colonies.	Specimens	% of total	No. of colonies.	Specimens	% of total	No. of colonies.	Specimens	% of total	No. of colonies.
1	59	16·6		36	12·75		7	8·5		28	9·9	
2	47	13·2	9	40	14	8	22	6·1	10	16	5·67	3·54
3	29	8		38	13·5		21	5·9		10	3·54	
4	28	7·9	10	41	14·5	11	23	6·4	7	7	2·48	0·7
5	33	9·3		29	10		18	5		7	2·48	
6	33	9·3	12	28	9·9	Total	22	6·1	282	2	0·7	100
4 or less	163	46		155	55		355	100		282	100	

It is evident from Table 3 that *T. mentagrophytes* is isolated in high colony numbers more often than *T. rubrum*. These isolations from all, or almost all, twelve inocula could be a result of a dense hyphal development of *T. mentagrophytes* in the absence of host reaction. The figures for *T. rubrum* represent more or less what might be expected in a series of isolations from material when the amount of fungus present varies only slightly from specimen to specimen.

II. HUMAN HAIR SPECIMENS

The results obtained by the method described above were entirely satisfactory, but it is by no means implied that isolation of dermatophytes from all types of clinical material can be carried out in this way. It is generally accepted, however, that isolations are usually more easily obtained from infected human hair than from skin, and the above method therefore needs no modification for use with such material. In hair the dermatophytes are generally present in large quantity and, if small fragments of carefully selected material are used as inocula, little difficulty is experienced.

III. ANIMAL SPECIMENS

Because of the much higher rate of mould contamination, isolation from animal material is much more difficult and special precautions may be necessary. It is our experience, however, that successful isolations are often obtained by the method already given, if the material is carefully selected and the inocula are reduced to minimum size. It is advisable, however, to increase the number of inocula to twenty-four, or more. The number used in the isolations from man described above was governed by the size of the specimen and the necessity for adequate microscopical examination. With material from animals, limitation of method because of specimen size is the exception rather than the rule. We have found the lower 3 mm. of hairs cut to form four or five inocula is most profitable and many successful isolations from horse, cat and dog have been made on 4 % malt-extract agar using this portion of the specimens.

Bovine material requires particular mention since, in addition to very high numbers of contaminants which are present, the species of dermatophyte usually present (*T. verrucosum* var. *discoides*) develops very slowly on the commonly used media. The growth rate, and consequently the isolation rate, may be increased by adding the required vitamins to Sabouraud's dextrose agar (Georg, 1950) or by the use of nutrient agar as advocated by Austwick (1954). Once again, much can be done to offset the difficulties of contamination by careful selection of material and by using the smallest possible inocula. We have found the most fruitful source of infected material to be very small hair stubs within the waxy scales and these can readily be separated by pressure on the scale masses with the flat side of a scalpel blade.

In addition to the use of enriched media and the careful selection of inocula, however, some method of combatting mould contaminants is usually necessary. Austwick (1954) described a method involving spore

separation with which he obtained good results. Georg (1953) advocated the use of cycloheximide and also reported much improved results in isolation from both bovine specimens and human nails. We have evolved a method which relies for its success on the fact that many fungicidal preparations will kill fungi *in vitro* but are unable to penetrate tissue except after long periods of exposure.

Method

Selected material was divided into pieces large enough to give four inocula. These fragments were shaken in a test-tube containing an aqueous solution of the fungicide for a period of 5 min. The fungicide was then filtered off and the material transferred to a dry filter-paper where each piece was divided into four and inoculated, without washing, on slants of agar medium.

The results obtained with samples of cattle ringworm, using a fungicide containing mercury in organic combination, are shown in Table 4. The basal medium was the nutrient agar defined by Austwick (1954); the fungicide was used at the concentration recommended by the manufacturers for the treatment of athlete's foot.

Section A of the table shows that isolation from seven samples of the material was not possible on the basal medium, or on this medium containing cycloheximide (0.5 mg./ml.). *T. verrucosum* was isolated from only two samples (14 inocula) when penicillin (20 units/ml.) and streptomycin (40 units/ml.) were incorporated in the medium and from six of the seven samples (26 inocula) when the inocula had been previously washed in the fungicide. The contamination rate was high in all cases, and the selective action of the antibiotics was obvious. When bacterial contaminants were reduced by the penicillin-streptomycin mixture, the moulds increased and the reverse was the case when cycloheximide was present alone. The fungicide reduced both types of contaminants. When material treated with the fungicide was inoculated on media supplemented with antibiotics, singly or in various combinations, the isolation rate was greater than that obtained by the use of antibiotics or the fungicide alone (section B of Table 4).

Georg (1953) reported that the number of dermatophytes isolated paralleled the degree of inhibition of the growth of saprophytic fungi on Sabouraud's dextrose agar supplemented with penicillin, streptomycin and cycloheximide, and that the addition of the antibiotics did not inhibit the growth of dermatophytes. This is not apparently true when nutrient agar is used. There were fewer contaminated inocula when the medium contained all three antibiotics than when one type only was present and the inocula had been washed in the fungicide, but fewer positive inocula were obtained. Also, when the inocula were treated with the fungicide and all the antibiotics were present in the medium, there was no significant increase in the number of positive inocula, although the number of negative inocula was considerably higher than was the case with fungicidal treatment and one type of antibiotic (Table 4 section, B). It is probable that the fungicide also inhibits or retards dermatophyte development to some extent.

Table 4. Comparison of isolation rates on a basal medium with variation of supplement and treatment of inocula

Specimen no.	Section A			Section B		
	Basal medium. Control			Basal medium plus penicillin and streptomycin		
	Pos. inocula	Neg. inocula	Contaminated inocula	Pos. inocula	Neg. inocula	Contaminated inocula
1	0	0	24	13	0	11
2	0	0	24	1	3	20
3	0	0	24	0	0	24
4	0	0	24	0	0	24
5	0	0	24	0	0	24
6	0	0	24	0	0	24
7	0	0	24	0	0	24
Totals	0	0	168	14	3	151
Specimen no.	Basal medium plus cycloheximide			Basal medium plus penicillin and streptomycin. Inocula washed in fungicide		
	Pos. inocula	Neg. inocula	Contaminated inocula	Pos. inocula	Neg. inocula	Contaminated inocula
	Pos. inocula	Neg. inocula	Contaminated inocula	Pos. inocula	Neg. inocula	Contaminated inocula
1	11	4	9	19	4	1
2	7	8	9	8	10	6
3	1	1	22	1	7	16
4	4	2	18	8	6	10
5	2	2	20	1	11	12
6	1	2	21	3	2	19
7	0	0	24	2	2	20
Totals	26	19	123	42	42	84
Specimen no.	Basal medium plus penicillin, streptomycin and cycloheximide. Inocula washed in fungicide			Basal medium plus penicillin, streptomycin and cycloheximide. Inocula washed in fungicide		
	Pos. inocula	Neg. inocula	Contaminated inocula	Pos. inocula	Neg. inocula	Contaminated inocula
	Pos. inocula	Neg. inocula	Contaminated inocula	Pos. inocula	Neg. inocula	Contaminated inocula
1	11	13	0	22	2	1
2	11	12	1	9	14	0
3	5	19	0	3	8	16
4	6	17	1	2	9	9
5	1	23	1	1	11	7
6	7	16	0	0	0	14
7	6	17	1	3	5	3
Totals	47	117	4	40	49	50

The incubation temperature was 35° C. and the first isolates of *T. verrucosum* could be identified after 6–7 days. This result confirms the value of the nutrient agar for the isolation of *T. verrucosum* and compares favourably with the incubation period required by Austwick (1954), on the same medium, and Georg (1950), on vitamin-enriched Sabouraud's dextrose agar. The results given in Table 4 are those obtained after 12 days' incubation.

Table 5.* Comparison of the effect on the isolation rate of treatment of inocula with different types of fungicides

	Basal medium. Control	Basal medium. Inocula washed in fungi- cide I	Basal medium. Inocula washed in fungi- cide II	Basal medium. Inocula washed in fungi- cide III	Basal medium. Inocula washed in fungi- cide IV	Basal medium. Inocula washed in fungi- cide V
Positive inocula	2	16	10	3	14	19
Negative inocula	0	8	0	0	10	5
Contaminated inocula	22	0	14	21	0	0
Totals	24	24	24	24	24	24

* These results show that a wide range of fungicidal substances may be used in the manner described in the text, and are not in any way intended as an indication of the suitability or efficiency of the products for use in treatment of infection.

A further experiment (see Table 5) was carried out to compare the effect on the isolation rate of various types of fungicidal preparations. The products were used at the concentration recommended by the manufacturers for treatment of ringworm infections with the exception of no. III which was used at the concentration recommended for use in prophylaxis. This may account, in some measure, for the failure of this product to reduce contamination; it is possible that a higher concentration would have been more effective.

The products contained the following active agents: mercury in organic combination (I); formalin and sodium pelargonate (II); a surface ampholytic compound (III); a di-chlorophene compound (IV); a quaternary ammonium salt (V).

The specimen, which was again from a bovine infection, was less heavily contaminated with moulds than those used in the previous tests and the results are therefore not directly comparable. It is evident, however, that many types of products can be used in this way; some are more effective than others, but variation of concentration or exposure time may improve the efficiency of any one.

The method is not confined to use with bovine material. Successful isolations of *T. mentagrophytes*, *T. rubrum* and *Microsporum canis* from human and animal material have been obtained on a variety of culture media after treatment of the inocula with a fungicide in the manner described.

The advantages of the technique are that the cost is negligible, it can be used with any medium and the dermatophytes isolated are typical in appearance for the particular medium employed. It has the disadvantage that preparation for culture is a rather more lengthy process than usual. On the other hand, much less time is required for the process than for the

note that there are no antibodies in this series

preparation of media containing antibiotics, if, as is often the case, such media are not immediately available. The pretreatment of inocula with a fungicide, in addition to the use of a medium supplemented with antibiotics when it is available, will in many cases lead to isolation from a specimen too badly contaminated to yield a culture by antibiotic action alone.

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THE CLAVARIACEAE OF THE MUSSOORIE HILLS (INDIA). II

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(With Plate 20 and 7 Text-figures)

Five new species and two new varieties of Clavariaceae are here described: *Ramaria flaviceps* sp.nov., *R. flaviceps* var. *cremea* var.nov., *R. clarobrunnea* sp.nov., *R. camelicolor* sp.nov., *R. camelicolor* var. *rufa* var.nov., *Clavulina hispidulosa* sp.nov., *Clavaria amoenoides* sp.nov.

Type collections have been deposited in the herbarium of the Panjab University. Duplicate material, in alcohol-formalin, is at the Botany School, Cambridge.

INTRODUCTION

Under the leadership of Prof. P. N. Mehra an excursion is made every year from the Botany Department of the Panjab University to the Mussoorie Hills in the North-Western Himalayas (5000-7000 ft. altitude) to study the cryptogamic flora. The Clavariaceae are part of the programme undertaken by Dr K. S. Thind and his students. The first contribution (Thind & Anand, 1955) describes seven known species with one new variety. This second contribution deals with five new species and two new varieties. The fruit bodies have been described from fresh material, supplemented with dried material and that preserved in alcohol-formalin.

Fleshy fungi are recognized most easily from their living, but evanescent characters. The problem is to identify them with the herbarium-species for which only microscopic characters may be reliable. It is known that many species, unmistakable in the field, appear almost identical in the herbarium. Thus, of the species here described, *Clavaria amoenoides* is indistinguishable in the dried state from *C. fumosa*, and *Ramaria flaviceps* can be matched with dried material (lacking field-notes) from Europe and North America. As we are still so deficient in knowledge of the field characters of these fungi and of the variability of widespread species, our policy is to describe as new what we can distinguish as living entities.

The classification of Corner (1950) has been followed.

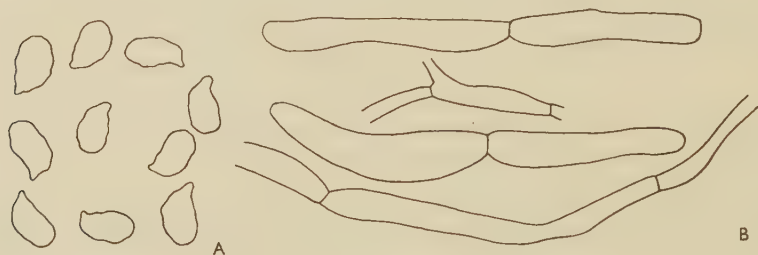
The numbers of the species are the serial numbers of the flora.

8. *Ramaria flaviceps* sp.nov. var. *flaviceps* (Plate 20, fig. 1; Text-fig. 1)

Receptacula ad 7.8 cm. alta, parviuscula, dichotoma, spurie polychotoma, alba, apicibus luteis: trunco ad 3 × 0.8 cm.: carne alba: sporis 7-10.5 × 4-5.3 μ, brunneolis, subverrucosis: hyphis 3-18 μ latis, haud fibulatis. *R. flavae* affinis, sporis multo minoribus.

Fructifications up to 7.8 cm. tall and 2.5 cm. wide, gregarious, rarely caespitose, erect, rather small, radial, glabrous, smooth, *white, the branch-tips deep yellow* for a distance of 0.5 cm.: trunk to 3×0.8 cm., cylindrical: branches dichotomous, appearing polychotomous above through close dichotomy, the lower internodes long and thick, the upper thin and small or inconspicuous, the deep yellow apices blunt and appearing polychotomous through close dichotomy: flesh white, unchanging: taste bitter or inparticular: smell inparticular.

On soil in oak forest (*Quercus incana* Roxb.): India (Dhobi Khad, Mussoorie, 7 August 1953, No. 36).



Text-fig. 1. *R. flaviceps* sp. nov. A, spores, $\times 880$; B, hyphae, $\times 380$.

Hymenium amphigenous, thickening to 70μ . *Basidia* $4-9\mu$ broad, clavate: sterigmata 4, $3-7\mu$ long, slightly incurved. *Spores* $7-10.5 \times 4-5.3\mu$ (overall), light brown, broadly ellipsoid, rough or inconspicuously verrucose, aguttate, the spore contents sometimes contracted as a gutta. *Hyphae* monomitic, $3-18\mu$ wide, the cells $35-102\mu$ long, *without clamps*, thin-walled, branched, hyaline, inflated, usually constricted at the septa, without secondary septa.

This is one of several species closely allied with *R. flava* (Fr.) Quél., but differing in the much smaller spores and unchanging flesh. Distinctive of the alliance is the absence of clamps.

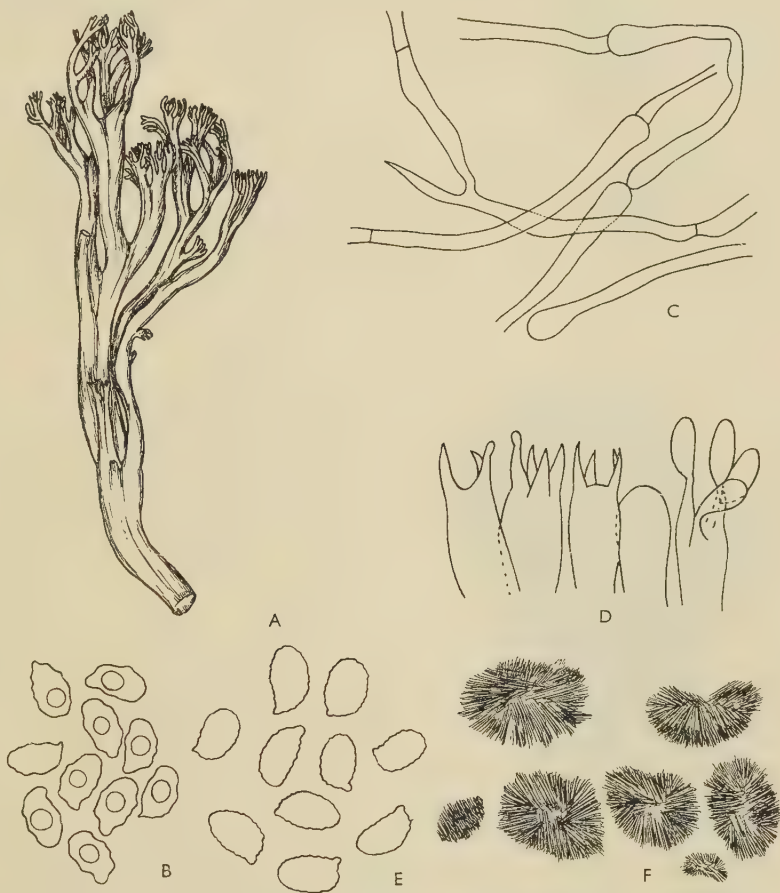
9. ***Ramaria flaviceps*** Corner, Thind & Anand var. ***cremea*** var. nov.
(Pl. 20, fig. 2; Text-fig. 2)

A typo differt receptaculis ex integro cremeis vel superne brunneolis, apicibus ramulorum haud luteis, sporis latoribus, $5-7\mu$ latis, verrucis planis distincte adornatis.

Fructifications up to 10 cm. tall and broad, solitary, erect, medium to large, radial, without a trunk, profusely branched, fleshy, smooth, glabrous, *cream-coloured or whitish below and light brown above*: branches dichotomous, unequal, in alternating planes, rather compact, internodes long below and shorter above, primary branches to 1.5 cm. wide, ultimate branchlets minute or to 2 mm. long, in pairs or cristate through close dichotomy, *the apices blunt and concolorous*: flesh whitish or paler concolorous, unchanging: taste bitter to inparticular: smell inparticular.

On soil in oak forest: India (Chakrata Toll, Mussoorie, 6 August 1953, No. 37, *typus*; Dhobi Khad, Mussoorie, 10 September 1953, No. 38).

Hymenium amphigenous, thickening to 88μ . *Basidia* $7-10\mu$ broad, clavate, brown: sterigmata (2-3-)4, $3.5-7\mu$ long, straight or slightly incurved. *Spores* $6-11 \times 5-7\mu$ (overall), light brown, broadly ellipsoid, distinctly verrucose with small flat warts, aguttate or uniguttulate, the guttule filling one-quarter to one-third of the spore-cavity. *Hyphae* $4-8\mu$ wide, to 18μ wide at the vesicular swellings, without clamps, thin-walled hyaline, branched, inflated, often with vesicular swellings terminal or on one side of the septum, long-celled, without secondary septa.



Text-fig. 2. *R. flaviceps* Corner, Thind & Anand var. *cremea* var. nov. (A-C, No. 37; D-F, No. 38.)

A, $\times 1$; B, spores, $\times 880$; C, hyphae, $\times 380$; D, basidia, $\times 880$; E, spores, $\times 880$; F, interhyphal masses, $\times 380$.

This variety has wider and more clearly marked spores than the type-variety and it lacks the yellow tips to the branches. It may be a distinct species, but there is evidence in herbaria of still more variation in colour and spore in this alliance, and we prefer to take a broad view of the relationships.

In No. 38, but not in No. 37, abundant interhyphal masses were observed (Text-fig. 2F). They were soluble in dilute potash and were clearly

exudations of the hyphae, though the 'spines' were not crystalline, as in *R. camelicolor*. Such exudations may well be distinctive and should be looked for in living material: they are a caution against the exclusive use of potash for restoring preserved material for microscopic examination, excellent though it be.

10. ***Ramaria clarobrunnea*** sp.nov. (Text-fig. 3)

Receptacula ad 13×10.5 cm., multo-ramosa, magna, clarobrunnea, insipida, inodora, trunco ad 3×3 cm. bene evoluto, superne valde incrassato, ramis polychotomis superne dichotomis, carne subconcolori; sporis $9-11 \times 3.5-4.2 \mu$, pallide brunneis, ellipsoideis, subasperis, leniter spiraliter striatis: hyphis $3.5-11 \mu$ latis, fibulatis. *R. botrytidis* et *R. holobellae* affinis, color brunneo, nunquam rubescenti, diversa.

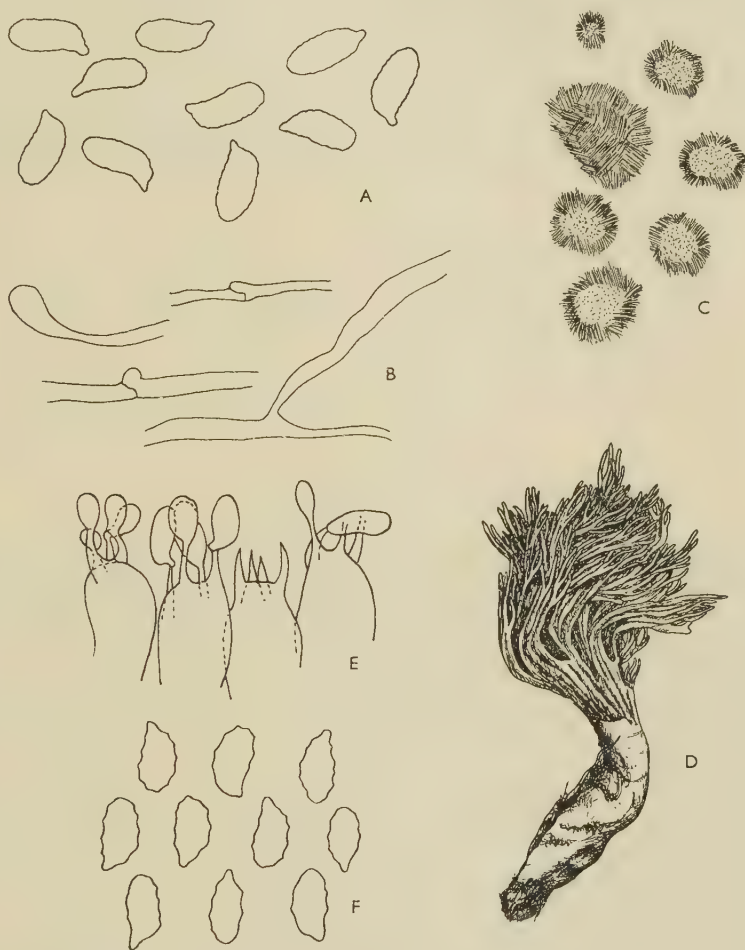
Fructifications up to 13×10.5 cm., solitary, erect, large, much branched, fleshy, brittle, smooth, glabrous, light brown: trunk to 3×3 cm., much thickened upwards, short, stubby, paler concolorous: branches polychotomous below, dichotomous above in alternating planes, cylindrical to slightly flattened, ultimate branchlets short and thick to 3 mm. long, the apices blunt and concolorous: flesh paler, concolorous, unchanging: taste and smell inparticular.



Text-fig. 3. *Ramaria clarobrunnea* sp.nov. A, $\times \frac{1}{2}$; B, basidia, $\times 880$; C, spores, $\times 880$; D, hyphae, $\times 380$.

On soil in oak forest: India (Dhobi Khad, Mussoorie, 21 August 1953, No. 39).

Hymenium amphigenous, absent from the trunk, thickening to 122μ . *Basidia* 6–9 μ broad, clavate: sterigmata, 4, 3·5–8·8 μ long, slightly incurved. *Spores* 9–11 $\times$ 3·5–4·2 μ (overall), pale brown, slightly rough and faintly



Text-fig. 4. *Ramaria camelicolor* sp. nov. var. *camelicolor* (A–C, No. 40; D–F, No. 41.) A, spores, $\times 880$; B, hyphae, $\times 380$; C, interhyphal masses, $\times 380$; D, $\times \frac{1}{2}$; E, basidia, $\times 880$; F, spores, $\times 880$.

spirally striate (the striations evident in dilute potash), aguttate. *Hyphae* monomitic, 3·5–11 μ wide, *clamped*, thin-walled, branched, hyaline, considerably inflated and at places with saccate swellings, narrowed in parts, septate at long intervals.

The faint spiral markings on the spores, especially evident in dilute potash, occur also in *R. botrytis* (Fr.) Rick, and *R. holorubella* (Atk.) Corner, and though *R. clarobrunnea* has the small spores of *R. holorubella*, it lacks the red-brown and vinaceous colours.

11. **Ramaria camelicolor** sp. nov. var. **camelicolor** (Pl. 20, fig. 3;
Text-fig. 4)

Receptacula ad 14×10.4 cm., multoramosa, magna, pallide camelicoloria vel alutacea, insipida, inodora, trunco ad 4×1.8 cm., saepe maliformi, ramis polychotomis, superne dichotomis vel ex integro dichotomis, carne concolori: sporis $9-13 \times 4.5-6 \mu$, brunneis, ellipsoideis, minute verrucosis: hyphis fibulatis, $4-10 \mu$ latis.

Fructifications to 14×10.4 cm., solitary, erect, *large*, usually massive, radial, *profusely branched*, fleshy, smooth, glabrous, *very light camel-brown or buff-coloured*: trunk to 4×1.8 cm., often undifferentiated or as a stubby basal portion to 6.5 cm. wide, paler concolorous: branches polychotomous below, dichotomous above, or dichotomous throughout in less massive



Text-fig. 5. *Ramaria camelicolor* Corner, Thind & Anand var. *rufa* var. nov.
A, basidia, $\times 880$; B, spores, $\times 880$.

fruit-bodies, in alternating planes, rather compact, primary branches to 1.3 cm. broad, ultimate branchlets mostly as minute protuberances but some 2-5 mm. long and paired or crowded through close dichotomy, the apices blunt and concolorous: flesh concolorous or paler, unchanging: taste and smell inparticular.

On soil in oak forest: India (Dhobi Khad, Mussoorie, 28 August 1953, No. 40, *typus*): in pine-oak forest, India (Dhobi Khad, Mussoorie, 29 July 1953, No. 41).

Hymenium amphigenous, usually absent from the trunk, thickening to 105μ . *Basidia* $7-11 \mu$ broad, clavate: sterigmata 4, $3.5-7 \mu$ long, straight or slightly incurved. *Spores* $9-13 \times 4.5-6 \mu$ (overall), light brown to brown, ellipsoid, minutely but distinctly verrucose, aguttate. *Hyphae* monomitic, $4-10 \mu$ wide, the cells $84-120 \mu$ long or more, *clamped*, thin-walled, branched, hyaline but brownish in the mass, not inflated.

As in *R. flaviceps* var. *cremea*, abundant interhyphal masses, soluble in dilute potash, were found in one collection (No. 40) but not in the other (No. 41), and the 'spines' were distinctly crystalline.

The spores of No. 41 were also slightly larger ($10.5-13 \times 4.6-5.3 \mu$) than in No. 40 ($8.8-11.2 \times 4.2-6 \mu$). Yet the two agree in all other respects. The species may be widely distributed in Asia, because there is a collection from Indonesia, represented by a painting (No. 1702 Herb. Hort. Bogoriensis) without specimen, which agrees in shape and colour and, if one may judge from the painting, with similar verruculose spores *c.* $10-12 \times 4-5 \mu$. *R. madagascariensis* may be allied. Distinctive are the large, pale brownish-buff fruit-bodies with unchanging flesh and verruculose spores.

12. **Ramaria camelicolor** Corner, Thind & Anand var. **rufa**
var. nov. (Pl. 20, fig. 4; Text-fig. 5)

Receptacula pallide rufa: sporis $7.7-10.5 \times 5.3-7 \mu$.

Fructifications to 13×7.5 cm., solitary, erect, medium to large, radial, without a trunk, profusely branched, fleshy, smooth, glabrous, faintly red, the base whitish for a length of 3.5 cm.: branches polychotomous below, dichotomous above, compact, unequal, in alternating planes, primary branches long and to 1 cm. wide, often joined, the ultimate branchlets minute and crowded or cristate through close dichotomy, the apices blunt and concolorous; flesh concolorous: taste and smell inparticular.

On soil in oak forest; India (Dhobi Khad, Mussoorie, 21 August 1953, No. 42).

Hymenium amphigenous, absent from the white base, thickening $88-158 \mu$. *Basidia* $8-11 \mu$ broad, clavate: sterigmata 4, $4-7 \mu$ long. *Spores* $7.7-10.5 \times 5.3-7 \mu$ (overall), pale brown, ellipsoid, in some cases broadly, verrucose, uniguttate, the gutta almost filling the spore-cavity. *Hyphae* monomitic, $3-11 \mu$ wide, clamped, hyaline to slightly coloured, thin-walled, branched, not or slightly inflated, septate at long intervals.

This fungus resembles *R. camelicolor* so closely that it is treated merely as a variety.

13. **Clavulina hispidulosa** sp. nov. (Text-fig. 6)

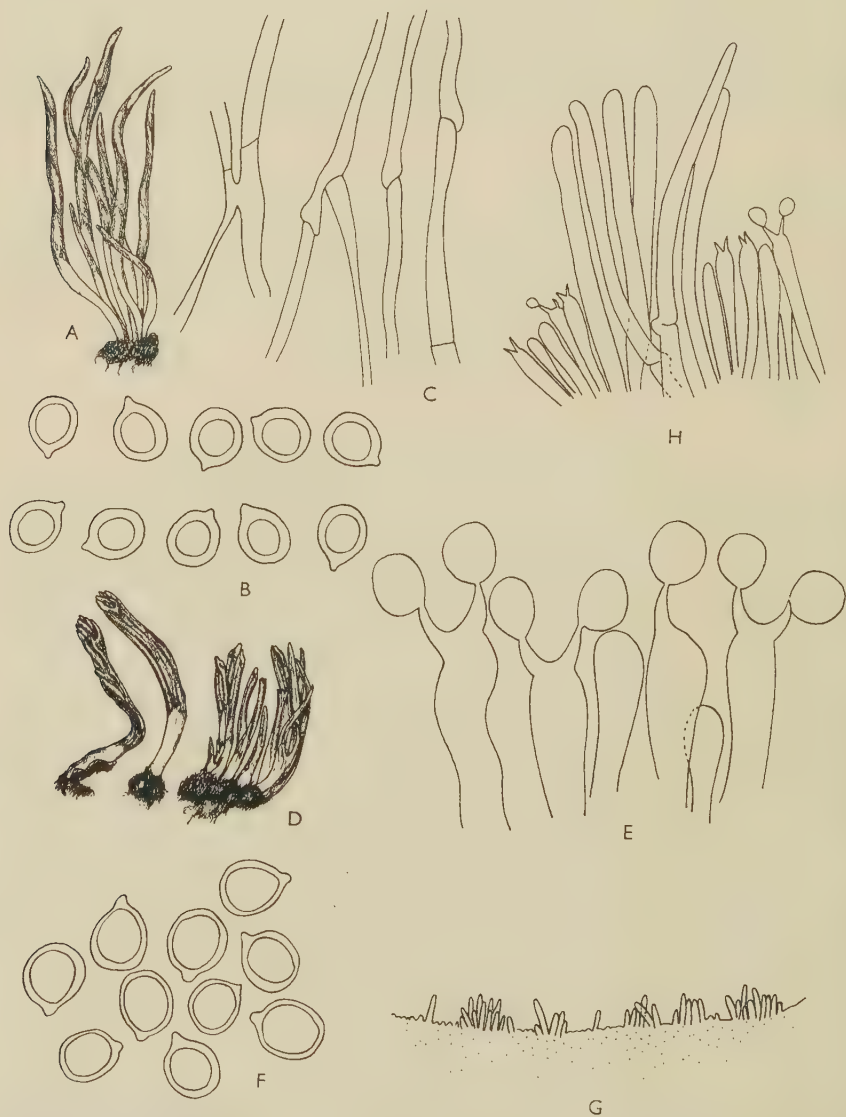
Receptacula simplicia ad 11×0.8 cm. vel plus minus ramosa, solitaria, gregaria vel caespitosa, juventute albida, dein grisea vel fuliginea, aetate saepe fistulosa, insipida, inodora: trunco sterili saepius angustiori: ramis diversis, sparsis vel numerosis, irregularibus, saepe sulcatis et connatis: hymenio hyphis sterilibus, $5-8 \mu$ latis, levibus, cylindricis, septatis, obtusis, $5-25$ fasciculatis, raro solitariis, ad 120μ projicientibus hispiduloso: sporis $8-12 \times 7-10.5 \mu$ subglobosis vel ovoideis, 1-guttatis: sterigmatibus (1-) 2: hyphis $2-14 \mu$ latis, fibulatis. *C. léveilléi* affinis, hymenio hyphis fasciculatis hispiduloso diversa.

Fructifications simple, to 11×0.8 cm., or variously and irregularly branched, solitary, gregarious or caespitose in clusters to 1.5 cm. wide, erect, radial, sometimes flattened, fleshy, at first white, then grey to fuliginous: trunk usually narrower than the fertile head, paler, to 5 cm. long, usually one-third or less of the fruit-body: fertile clubs solid or becoming hollow with age, often flattened and longitudinally grooved, often 2-3 connate: branches sparse to abundant, irregular, radial or flattened and mostly grooved, often connate, solid or hollow with age, as short apical proliferations or more or less basal and resembling the simple clubs, the apices acute or blunt, concolorous; flesh concolorous or paler: taste and smell inparticular: abundant basal white mycelial strands.

On humus-soil in oak forest; India (Dhobi Khad, Mussoorie, 10 September 1953, No. 43, *typus*; Chakrata Toll, Mussoorie, 2 September 1953, No. 44).

Hymenium amphigenous, absent from the trunk, thickening to 140μ with numerous embedded spores, minutely hispid from tufts of emergent, thin-walled, smooth, unbranched, blunt, cystidium-like hyphae $5-8 \mu$ wide, in fascicles of $5-25$, rarely solitary, projecting to 120μ beyond the hymenial surface, continued below into the subhymenium with septate

and clamped stalks (thus persistent as sterile emergent hyphae during the thickening of the hymenium), the fascicles absent from the trunk. *Basidia* 7–10.5 μ broad, clavate: sterigmata 2, or 1–2, 3.5–10.5 μ long, usually straight. *Spores* 8–12 $\times$ 7–10.5 μ , subhyaline to pale yellow or pale brownish, subglobose to obovoid, smooth with one large gutta filling about one half of the spore-cavity. *Hyphae* monomitic, 3.5–14 μ wide, the cells 26–150 μ



Text-fig. 6. *Clavulina hispidulosa* sp. nov. (A–C, No. 43; D–H, No. 44.), A, simple fructifications, $\times \frac{1}{2}$; B, spores, $\times 880$; C, hyphae, $\times 380$; D, simple and slightly branched fructifications, $\times \frac{1}{2}$; E, basidia, $\times 880$; F, spores, $\times 880$; G, hispid hymenium with tufts of emergent hyphae, $\times 190$; H, a tuft of emergent hyphae with subhymenial connexions, $\times 380$.

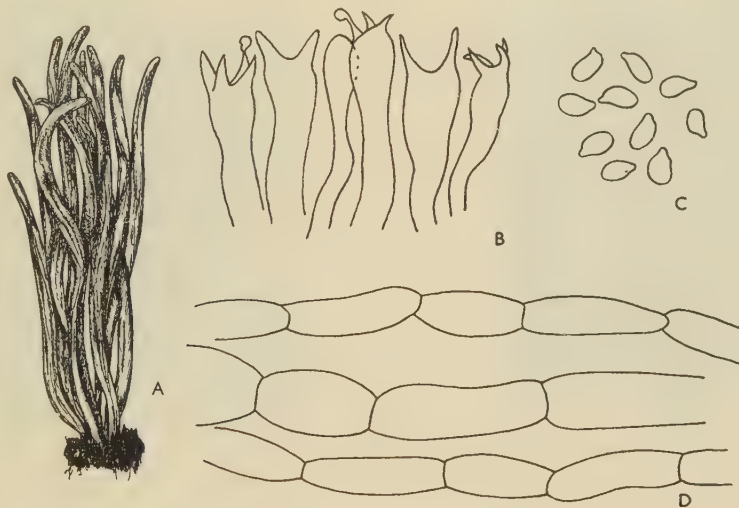
long or more, clamped, thin-walled, branched, hyaline, inflated, a few narrow and uninflated with distant septa, H-connexions present.

At first sight, this species seems to come between *C. rugosa* and *C. cinerea*, but the fascicles of hyphae in the hymenium are characteristic and, as they occur also in some Australian species which have yet to be properly described, they seem to indicate a group of Australasian species, to which *C. léveilléi* may also belong.

14. *Clavaria amoenoides* sp.nov., subgen. Syncoryne (Text-fig. 7)

Receptacula ad 12×0.6 cm., simplicia, solitaria, gregaria vel caespitosa, pallide aurantia, stipiti ad 3.3×0.4 cm. pallidiori, inodora, insipida: sporis $6-7 \times 3-4 \mu$, ellipsoideis, aguttatis: basidiis haud fibulatis, sterigmatibus 2-3-4: hyphis ad 25μ latis, breviter septatis, aliis angustis distanter septatis intermixtis, haud fibulatis.

Fructifications to 12×0.6 cm., *simple*, solitary, gregarious or caespitose in clusters up to 2 cm. broad, erect, medium-sized, radial, fleshy, firm, smooth, glabrous, *very pale orange-yellow*: trunk to 3.3×0.4 cm., narrower than the fertile head, paler concolorous: fertile head cylindric, sometimes twisted and longitudinally grooved, solid, obtuse, the apex concolorous: flesh concolorous, unchanging: taste and smell inparticular.



Text-fig. 7. *Clavaria amoenoides* sp.nov. A, simple and caespitose fructifications, $\times \frac{1}{2}$; B, basidia, $\times 880$; C, spores, $\times 880$; D, hyphae, $\times 380$.

On soil in pine-oak forest: India (The Park, Mussoorie, 11 September 1953, No. 45).

Hymenium amphigenous, absent from the trunk, thickening to 105μ . *Basidia* $4.2-7 \mu$ broad, clavate, *without clamps*: sterigmata 2, 3 or 4, $3.5-5.3 \mu$ long, usually straight. *Spores* $6-7 \times 3-4 \mu$, subhyaline, obovoid, to broadly ellipsoid, smooth, aguttate. *Hyphae* monomitic, to 25μ wide, mostly with short cells $35-103 \mu$ long and often with oblique ends, some of

the branches narrow and uninflated with distant septa, *without clamps*, thin-walled, hyaline, parallel, compact, secondary septa apparently absent.

Such a simple, yellow clavaria suggests *Clavulinopsis amoena* (Zoll. et Mor.) Corner, but the spores, the short sterigmata, and the total absence of clamps show that it is a true *Clavaria* s.st. Indeed, the preserved specimens are practically indistinguishable from *Clavaria fumosa* and *C. vermicularis*. This species, in fact, must put one on guard against identifications without colour notes and, alternatively, without microscopic study.

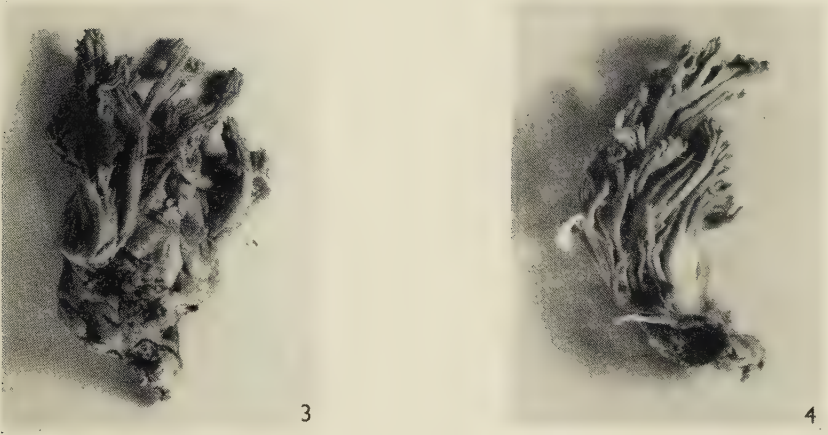
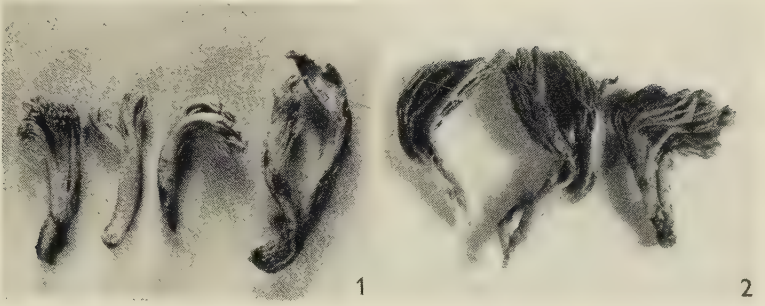
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EXPLANATION OF PLATE 20

- Fig. 1. *Ramaria flaviceps* sp.nov. var. *flaviceps* (No. 36).
Fig. 2. *R. flaviceps* Corner, Thind & Anand var. *cremea* var.nov. (No. 38).
Fig. 3. *R. camelicolor* sp.nov. var. *camelicolor* (No. 40).
Fig. 4. *R. camelicolor* Corner, Thind & Anand var. *rufa* var.nov. (No. 42).

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FUNGI OCCURRING IN MIXED OAKWOOD AND HEATH SOIL PROFILES

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The active fungi occurring in a brown forest soil and in a podzol soil developed from the former following replacement of the mixed oakwood cover by *Calluna* were studied seasonally, employing the screened immersion-plate technique.

More mycelia were isolated from the podzol soil than from the brown forest soil and the B₁ horizon of the former was the most favourable horizon for growth, particularly for *Mucor ramannianus*.

Maximum growth was obtained in summer and least in winter, in both soils.

Over fifty species were isolated, the most common being, *Trichoderma viride* Tribes I and II, *Mucor ramannianus*, *Penicillium frequentans*, *Mortierella humilis*, *M. gracilis*, *M. nana* and *M. stylospora*.

Soil fungal patterns, based on percentage frequency of occurrence, are shown for both soils and indicate that the change in vegetation and soil type modified the basic brown forest soil fungal pattern.

INTRODUCTION

So far as the ecology of soil-inhabiting fungi is concerned investigations of cultivated soils are rather limited because of the inability to define precisely the operative factor in any individual demonstrable change of micro-population. In cultivated soils the diverse treatments, both mechanical and chemical, associated with the farming routine and the successive alternation of the vegetative cover and of root systems must influence the microflora of these soils to varying degrees. In contrast, natural or semi-natural vegetation covers soils in which any changes are gradual and in which the soil microflora is in dynamic equilibrium with its environment. Using appropriate techniques it should be possible to determine that section of the fungus flora which is active under particular environmental conditions. Changes in environment might be expected to bring about changes in the fungus flora of the underlying soils and the accumulation of such evidence should contribute to an understanding of the ecology of soil-inhabiting fungi. To this end soils derived from Bunter sandstone near Nottingham, carrying different stable and relatively persistent vegetations, were investigated by the screened immersion-plate technique (Thornton, 1952), in an attempt to discover any relationship between soil type, vegetative cover and active soil fungal populations. The natural vegetation of these soils is either mixed oakwood or heath (Hopkinson, 1927), and several horizons within a brown forest soil and a podzol soil under such respective vegetations were jointly examined at seasonal intervals over a period of 10 months.

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MATERIALS AND METHODS

Soils and vegetation

The Bunter sandstone is mainly quartz and contains only small quantities of other minerals such as orthoclase and mica and little, if any, apatite (Black, 1950). The soils have little structure, are coarse grained, and the low fertility is intensified by the rapid percolation of rain water leaching plant nutrients.

The original vegetation of the Bunter sandstone in north Nottinghamshire was an oakwood forest (Sherwood Forest) of which only a few areas exist at the present time. Such remnants have a tree canopy of *Quercus robur* L., *Q. petraea* (Matt.) Liebl. and hybrids, which locally assume dominance according to prevailing conditions. The ground vegetation of *Pteridium aquilinum* L., *Deschampsia flexuosa* (L.) Trin. and *Holcus mollis* L. may be replaced over larger or smaller areas by *Calluna vulgaris* (L.) Hull. Two localities were selected within half a mile of one another with different ground vegetation and different soil profiles. Soil I, in Thieve's Wood, carried a ground vegetation of *Aira* and *Holcus* under a *Quercus* canopy over a typical brown forest soil profile: soil II, in Barber's Wood, a ground vegetation of *Calluna* over a podzol profile. Details of these profiles are given in Table 1.

Table 1. *Descriptions of soil profiles on Bunter sandstone*

Horizon	Depth (in.)	Description
Soil I. Brown forest soil under <i>Quercus</i> , <i>Deschampsia flexuosa</i> and <i>Holcus mollis</i>		
A ₀₀	1	Leaf and litter layer
A ₀	$\frac{1}{2}$	Dark humified organic matter mixed with bleached sand grains
A	4	1-1½ in. grey brown, semi-compact, silty sand over 2½-3 in. brownish, semi-compact, silty sand
B	8	Orange-brown compact sand with pebbles, coloration decreasing with depth, transitional to C
C	On	Light yellow compact sand
Soil II. Podzol soil under <i>Calluna vulgaris</i>		
A ₀₀	$\frac{1}{2}$	Litter layer
A ₀	3-4	Humified litter and root debris mixed with sand grains
A ₂	6-8	Grey, semi-compact sand containing roots and pebbles up to 3 in. diam.
B ₁	2-3	Dark brown-black humified organic layer of compact sand containing pebbles
B ₂	6-8	Orange-brown compact sand, coloration decreasing with depth, transitional to C
C	On	Light yellow compact sand

Evidence suggests that the podzol has developed as a result of the decline in base supply to the soil surface due to the felling of the oakwood cover and its replacement by *Calluna*. The accumulation of an acid litter intensified the leaching of sesquioxides from the previously forested soil, leading to the development of a podzol profile. Lemee (1946) described a similar process occurring in France. The two soils are related to the extent that the podzol is a more leached stage of the brown forest soil which is

normal to the area. Using the screened immersion-plate technique the fungal status of these soils was examined for any differences which might be related to the change in vegetative cover.

Soil conditions

Details of soil conditions at the times of samplings are given in Table 2.

Maximum water-holding capacity was determined by a modification of Keen & Raczkowski's (1921) perforated-box method and values are given as the means of duplicate determinations. Organic carbon was determined by a wet-combustion method devised by Walkley and Black (see Piper, 1950) and the results given are the means of duplicate determinations.

Table 2. *Physical and chemical conditions in Bunter sandstone soils*

Horizons ...	Soil I			Soil II			
	A	B	C	A ₂	B ₁	B ₂	C
Maximum water holding capacity	30.2	35.1	21.2	22.0	53.7	30.1	26.8
Organic carbon (Walkley & Black values)	0.89	0.74	0.04	0.70	3.24	0.71	0.09
pH (glass electrode)	5.1	5.0	5.2	5.9	4.8	5.1	5.2
Soil moisture % (D.W. basis)							
Summer	11.9	13.7	4.6	6.2	20.3	11.3	4.4
Autumn	15.5	16.3	8.0	8.4	23.8	12.7	6.8
Winter	14.6	12.5	7.3	11.3	20.9	20.1	10.0
Early spring	15.7	18.4	8.0	12.9	26.2	26.1	9.2
Spring	13.3	18.0	9.6	12.7	24.7	27.8	10.3
Soil temperature (° C.)							
Summer	13.3	13.3	12.2	14.4	14.4	13.3	12.2
Autumn	8.3	9.0	9.1	8.2	8.5	8.9	9.1
Winter	3.3	3.3	3.9	3.2	3.3	3.4	3.9
Early spring	7.9	7.7	7.5	7.5	7.4	7.4	7.4
Spring	10.7	10.0	10.0	10.1	9.7	9.5	8.4

Soil reaction was measured with a Cambridge glass electrode pH meter and values are given as the means of several determinations made during the various seasons covered by the investigation.

Soil-moisture determinations were made on moist field soil weighed into crucibles, and dried in an oven at 110° C., to constant weight. Determinations were made at the beginning and the end of each observation period and moisture contents are expressed on a dry-weight basis as the means of these values.

Soil-temperature readings were taken at the beginning and the end of each sampling period with a thermometer pushed into the soil layers.

Sampling

Soil profiles were exposed by excavating a trench sufficiently deep to include the C horizon. Selected areas of such profiles were trimmed evenly with a metal blade, which was repeatedly sterilized with alcohol.

Screened immersion plates were placed in position in the manner already described (Thornton, 1952). Usually plates were placed with the long axis in a horizontal plane and one above another against one side of the trench except where pebbles prevented this strict order of placement. Plates were removed and sampled after incubation.

Five screened immersion plates were employed for each of seven soil horizons in soils I and II on each of five different sampling occasions.

Soils were sampled in the following approximate seasons: summer (7-17 July 1950), autumn (24 Oct.-7 Nov. 1950), winter (23 Jan.-13 Feb. 1951), early spring (24 Apr.-2 May 1951), and spring (18-28 May 1951), to provide seasonal observations on the soil fungal flora.

RESULTS

Results are presented in Tables 3 and 4 which show, respectively, the number of mycelia which were isolated from different soil horizons during different seasons, and the various species and the number of occasions they were isolated from different horizons over the period of study.

DISCUSSION

Apart from contributing to the knowledge concerning the active fungi inhabiting soils under particular vegetations, the main value of this investigation is thought to lie in the parallel study made of the fungal populations of two related and adjacent soils to provide comparisons which might discover any relationship between soil type, vegetation and active soil fungal populations.

Mycelia in soils

By recording the number of mycelia from beneath apertures of a number of screened immersion plates a quantitative measure of the relative distribution of mycelia in the two soils was able to be gained which may indicate the relative fungal status of these soils. The subsequent isolation,

Table 3. *Number of mycelia isolated from brown forest and podzol soil profiles*

Soil	Season					Total
	Summer	Autumn	Winter	Early spring	Spring	
Brown forest						
Horizon A	46	12	12	9	16	95
B	18	6	3	5	1	33
C	4	11	7	—	1	23
Total	68	29	22	14	18	151
Podzol						
Horizon A ₂	85	37	4	13	24	163
B ₁	112	51	2	4	18	187
B ₂	61	6	2	3	19	91
C	30	8	3	1	2	44
Total	288	102	11	21	63	485

Table 4. *Fungi isolated from oakwood and heath soil profiles by screened immersion plates*

Fungus	Soil I Brown forest			Soil II Podzol			
	A	B	C	A ₂	B ₁	B ₂	C
? <i>Chlamydomyces</i> sp.	1	.	.	.	.	.	.
<i>Mortierella marburgensis</i> Linm.	1	.	.	.	.	.	.
<i>Mucor hiemalis</i> Wehmer	2	.	.	.	.	.	.
<i>Penicillium piscarium</i> West.	2	.	.	.	.	.	.
<i>P. purpurescens</i> (Sopp) Thom & Raper	.	1	.	.	.	.	.
<i>Bisporomyces chlamydosporis</i> van Beyma	.	1	.	.	.	.	.
<i>Mortierella acuminata</i> Linm.	.	2	.	.	.	.	.
<i>Sporotrichum</i> sp.	.	.	1	.	.	.	.
<i>Paecilomyces</i> sp.	.	.	1	.	.	.	.
<i>Fusidium</i> sp.	.	.	2	.	.	.	.
<i>Trichoderma viride</i> Fr., Tribe II	12	6	2	.	.	.	.
Basidiomycete sp.	1	.	.	.	2	.	.
<i>Penicillium nigricans</i> (Bainier) Thom	1	.	.	1	.	.	.
<i>Mortierella minutissima</i> Linm.	3	.	.	.	1	.	.
<i>Penicillium notatum</i> West.	.	1	1	.	.	.	4
Sterile brown mycelium	5	1	1	.	.	1	.
<i>Mortierella vinacea</i> Dix.-Stew.	2	.	1	1	2	.	.
<i>Penicillium spinulosum</i> Thom	1	1	.	2	.	.	7
<i>Mortierella humilis</i> Linm.	10	3	2	2	1	.	1
<i>Penicillium melinii</i> Thom	1	1	1	1	1	1	.
<i>Mortierella gracilis</i> Linm.	3	3	1	26	29	13	2
<i>Trichoderma viride</i> Fr., Tribe I	15	1	4	44	48	14	4
<i>Penicillium frequentans</i> West.	5	3	1	21	39	15	8
<i>Zygorhynchus moelleri</i> Vuill.	1	2	.	9	1	4	2
Sterile white mycelium	4	2	.	2	1	1	.
<i>Mortierella nana</i> Linm.	3	7	.	1	2	1	.
<i>M. alpina</i> Peyr.	1	.	.	1	1	1	.
<i>M. stylospora</i> Dix.-Stew.	8	.	.	4	1	1	.
<i>Oidiodendron fuscum</i> Robak	.	.	2	3	1	.	1
<i>Cephalosporium acremonium</i> Corda	.	.	2	.	1	2	.
<i>Cylindrocarpon didymum</i> (Hartung) Wollenw.	2	.	.	2	.	1	.
<i>Mucor ramannianus</i> Moeller	.	.	.	27	50	24	5
<i>Mortierella elongata</i> Linm.	.	.	.	1	1	1	3
<i>M. polycephala</i> Coem.	.	.	.	4	.	1	1
<i>Penicillium thomii</i> Maire	.	.	.	2	.	1	1
<i>Mortierella exigua</i> Linm.	.	.	.	1	1	.	.
<i>M. jenkini</i> (Smith) Naumov.	.	.	.	.	1	3	.
<i>Penicillium implicatum</i> Biourge	.	.	.	2	.	.	1
<i>Verticillium</i> sp.	.	.	.	.	1	1	.
Yeasts	.	.	.	2	.	.	.
<i>Penicillium cyaneo-fulvum</i> Biourge	.	.	.	2	.	.	.
<i>Sporotrichum flavo-virens</i> Link	.	.	.	1	.	.	.
<i>Humicola grisea</i> Traaen	.	.	.	1	.	.	.
<i>Penicillium</i> sp. (decumbens series)	.	.	.	.	1	.	.
<i>Cephalosporium</i> sp.	.	.	.	.	1	.	.
<i>Gliocladium roseum</i> (Link) Bain.	.	.	.	.	.	1	.
<i>Penicillium waksmani</i> Zaleski	.	.	.	.	.	1	.
<i>Acrostalagmus albus</i> Preuss	.	.	.	.	.	1	.
? <i>Torula</i> sp.	.	.	.	.	.	1	.
<i>Hormodendron cladosporioides</i> (Fres.) Sacc.	.	.	.	.	.	1	.
<i>Penicillium stoloniferum</i> Thom	.	.	.	.	.	.	1
<i>Penicillium rubrum</i> Stoll	.	.	.	.	.	.	1
<i>Botrytis</i> sp.	.	.	.	.	.	.	1
<i>Trichobotrys</i> sp.	.	.	.	.	.	.	1

cultivation and identification of mycelia enabled an assessment of the relative distribution of fungus species to be made. Because isolations were made from mycelium in soil such species may be considered to play an important role in the biological processes of these soils. Comparisons of these related soils under seasonal conditions might be expected to supply useful information concerning soil fungus ecology.

The profile distribution of fungal isolations from soils I and II given in Table 3 provides a relative measure of the fungal status of these two soils. It would appear that the heath soil horizons had a higher active fungus population than forest soil horizons, allowing for the extra number of immersion plates buried in the former. Horizon B₁ in the heath soil provided a more favourable habitat for fungal growth, particularly for *Mucor ramannianus*, than any other horizon. Whereas the forest soil showed a decreasing fungal population with increasing depth in the profile, a general result in many investigations, the heath soil population exhibited an increase in the B₁ horizon relative to A₂ followed then by a decrease with depth. This was attributed to the high organic matter content of this horizon (see Table 2), and it supports the conclusions of Gray & McMaster (1933) and of Gray & Taylor (1935) that the activity of micro-organisms depends upon the organic matter relations of the various soil horizons.

An influence by season would appear to be reflected in isolations. (The period of incubation of screened plates in soil was increased in the colder seasons to allow for the slower growth rate of mycelium). In both profiles summer was the most favourable season for fungus growth, with a definite decline in autumn, to least growth in the colder seasons of winter and early spring followed by a recovery of activity in the spring. There is some conflicting evidence in the literature concerning the influence of season on the soil fungal population, although Szilvinyi (1948) concluded that both soil temperature and humidity influence soil micro-organisms and that fungi are first influenced by temperature. Eggleton (1938) suggested that seasonal changes in moisture and temperature are not the direct causes of the seasonal changes in microbial numbers, but that in controlling the growth of the herbage these climatic factors control the amount of energy material reaching the micro-organisms in the form of root excretions or of sloughed-off root material.

General nature of the population

In general terms, the present study confirms the features of the fungal population of acid forest and heath soils determined by a number of workers employing dilution-plating methods (Niethammer, 1935, 1937, 1939; Jensen, 1931; Cobb, 1932; Svinnufvud, 1937; Starc, 1941; Ellis, 1940; Rishbeth, 1951; Jefferys, Brian, Hemming & Lowe, 1953; and McLennan & Ducker, 1954). Johann (1932) considered Mucorales to be so characteristic of acid forest soils that he was able to distinguish various types of forest soils by the predominant mucoraceous fungi. Forest soils rich in bases (deciduous forests) contained an abundant population of the *M. flavus* type, somewhat more acid soils (mixed forest) contained the

M. ramannianus group and the very acid (raw humus forest) soils were characterized by a fungus flora of the *Zygorhynchus moelleri* type. In the brown forest soil studied here species of the genus *Mortierella* were most common, particularly *M. gracilis*, *M. humilis* and *M. nana*, while *Mucor hiemalis* and *Zygorhynchus moelleri* were also isolated. On the other hand, in the acid heath soil, although species of *Mortierella* were common, particularly *M. gracilis*, *Mucor ramannianus* was isolated in large numbers. Frequent occurrence of *M. ramannianus* in acid heath soil was found by Jefferys *et al.* (1953) and by McLennan & Ducker (1954). The former found that the distribution of *M. ramannianus* among nine profiles of acid heath soils was variable and appeared to be related to the occurrence of a well-marked iron pan in the B horizon. *Zygorhynchus moelleri* was also isolated by Jefferys *et al.* (1953) from heath soil but not by McLennan & Ducker (1954).

Except for three yeasts, which were not identified, members of the Ascomycetes were absent from immersion plates, although species of *Gymnoascus*, *Thielavia* and *Melanospora* appeared in dilution plates prepared at the same time.

Judged by the number of occasions on which mycelium with clamp connexions was isolated it would appear that members of the Basidiomycete group were not commonly present in mineral soil layers.

In common with other soil fungus studies the majority of species isolated belong in the Fungi Imperfecti. The genus *Penicillium* constituted an important group and the high incidence of *P. frequentans* in this study and in that of Jefferys *et al.* (1953) suggest that this fungus may possibly be regarded as characteristic of acid heath soils.

Species of *Trichoderma* have been considered so characteristic of soils that Okada (1938) suggested employing their occurrence as an index of the soil fungus population and of its distribution. Usually the species isolated have been referred to *T. lignorum* (Tode) Harz or *T. koningi* Oud. but Bisby (1939), after study of many specimens, concluded that such forms were the expressions of a single variable monotypic species, *T. viride*. In the present investigation two forms with green spore masses were isolated, one characterized by broad oval spores and the other by globose spores, which could be referred to the earlier species *T. koningi* and *T. lignorum*, respectively. Robak (1932), investigating the fungi attacking wood pulp, also recorded these two morphological forms. In the present study *T. viride* Tribe I refers to the form with globose spores and Tribe II to the form with oval spores. The separation of these two forms was considered to be important, because Tribe I was isolated from both soils whereas Tribe II was isolated only from the brown forest soil. This restriction suggests adaptation to particular habitat or substrate conditions which, if associated with particular and consistent physiological activities, may provide evidence for the retention of the earlier specific names.

A notable feature of this investigation was the absence of species of *Fusarium*. Jefferys *et al.* (1953) did not record them from heath soils, although McLennan & Ducker (1954) did. Niethammer (1941) found an association between species of *Fusarium* and species of *Mucor* and *Zygorhynchus* in similar soils.

Generally speaking the distribution of fungal species was fairly uniform throughout the profiles, apart from the decrease in numbers with depth. Approximately half the number of isolations of *Mucor ramannianus* and of *Penicillium frequentans* and one-third of the isolations of *Mortierella gracilis* were made from the B₁ horizon of the heath soil. Two-thirds of the isolations of *Trichoderma viride* Tribe II were made from the A horizon of the forest soil and so were the majority of specimens of sterile mycelium.

Soil fungal patterns

It is proposed to employ the term 'soil fungal patterns' for the species isolated by screened immersion plates arranged in decreasing order of percentage frequency of occurrence. Significance can then be attached to this section of floristic data because such fungi are actively taking part in the complexity of microbial activities in soil. The multiplicity of physical, chemical and biological factors, individually and in consort, which determine the types of microhabitats in particular soils also condition current growth and dormancy within those soils. As a result fungal patterns may be expected to vary with different soils and within the same soil under varying conditions. The more closely two soils approximate in their habitat characteristics the more closely their fungal patterns might be expected to resemble each other and vice versa.

The concept of patterns is employed in higher plant ecology where the flora of a prescribed area is characterized by the relative dominance and degrees of subdominance and frequency of occurrence of the various species growing in the area. It is necessary with higher plant investigations and with soil fungal investigations, to make regular and seasonal observations of the population in order to record all species occurring within a particular site. The screened immersion plates may be likened to the quadrats employed in higher plant ecology.

Lists of those species isolated on more than two occasions from the brown forest soil and of those isolated on more than three occasions from the heath soil are very similar. However, when soil fungal patterns are prepared differences in the fungal status between the two soils can be seen. The frequency of occurrence of the various fungal species in a particular soil is assessed as a percentage of the total number of isolations from that soil. Species included below are those contributing more than 5 % of total isolations from particular soils. This limit has been imposed for convenience in demonstrating the pattern concept.

Soil fungal patterns

Brown forest soil		Podzol soil	
	Percentage frequency of occurrence (approx.)		Percentage frequency of occurrence (approx.)
<i>Trichoderma viride</i> Tribe II	20	<i>Trichoderma viride</i> Tribe I	23
<i>T. viride</i> Tribe I	13	<i>Mucor ramannianus</i>	22
<i>Mortierella humilis</i>	10	<i>Penicillium frequentans</i>	17
<i>M. nana</i>	7	<i>Mortierella gracilis</i>	14
<i>Penicillium frequentans</i>	6		
<i>Mortierella stylospora</i>	5		

It would appear that the change in vegetation from mixed oakwood to *Calluna* on sandstone derived soils, in addition to initiating marked soil changes, brought about modifications in the basic pattern of the brown forest soil fungal flora. The occurrence of *Mucor ramannianus* in the heath soil in contrast to its absence in oakwood forest soil and the disappearance of *Trichoderma viride* Tribe II in the former, are the two most important changes, while there are alterations in the frequency of occurrence of yet other species.

The fact that a very small number of species contribute the greater bulk of mycelium suggests that in relatively undisturbed soils a small number of species assume dominance as a result of particular, favourable conditions.

It is felt that the screened immersion-plate technique and the concept of soil fungal patterns are capable of making useful contributions to the ecology of active, soil inhabiting fungi.

The potential activity of dormant spores should not be disregarded however.

The pattern approach could be extended to other groups of soil micro-organisms such as bacteria and protozoa, and through joint investigations, might lead eventually to the development of composite patterns, embracing several groups of micro-organisms, as a means of characterizing soil micro-populations and of assessing the influence of different conditions on these populations.

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STUDIES ON CEREAL MILDEW IN BRITAIN

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(With Plate 21 and 3 Text-figures)

Overwintering cleistocarps of *Erysiphe graminis* do not contribute to spring outbreaks of cereal mildew in this country. Ascospores, shed from July to late September, are the source of infection for successive crops of winter wheat and barley, but conidia from infected late tillers are the chief source for winter oats. Ascospore differentiation only takes place under high moisture, following the completion of a 'ripe-to-spore' stage. Optimum conditions for this are located at the base of the straws and the stubbles are the main source of the ascospore shower.

The intensity of the shower in any season is related to the environmental conditions during the growth of the crop and the degree of humidity at and following harvest. Direct infection of autumn-sown cereals may occur but more generally it derives from stubble volunteer seedlings infected by the ascospores. The timing of the ascospore shower and the extent to which volunteer seedlings develop on the stubbles determine the incidence of mildew in early-sown winter cereals, but susceptibility to disease decreases as the date of sowing is delayed. Although existing lesions continue growth and sporulation, during the winter there is little further spread of infection through the crops.

The incidence of infection foci for the build-up of mildew in spring is thus dependent upon the climatic conditions prevailing during the previous year and the extent and timing of the autumn cultivations.

Much is known of the host-parasite relationship in cereal mildew epidemiology but less of the significance of the sources of infection. This investigation has been made into the contribution which these make to the seasonal outbreaks of mildew in this country.

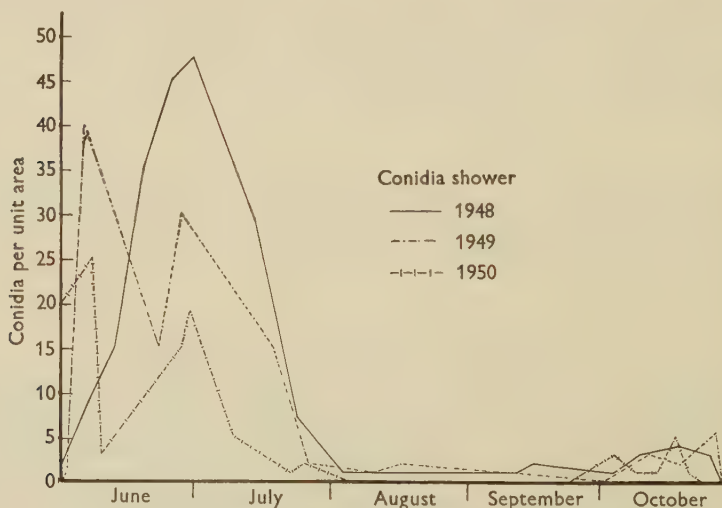
ASCOSPORES AS A SOURCE OF INFECTION

Cereal mildew infection takes place in spring and autumn. Two sources are possible for the spring outbreaks, ascospores from overwintering cleistocarps and conidia from infected autumn-sown crops. Cherewick (1944) and Foster & Henry (1937) record both methods as occurring in N. America, but in Europe (Honecker, 1936; Pape & Rademacher, 1934), in India (Mehta, 1930), and in the U.S.S.R. (Gorlenko, 1940), spring infection is thought to derive from overwintering plants and this is the generally accepted source for this country. The cleistocarps on English cereals usually contain undifferentiated asci at harvest. Some workers consider them to be largely abortive but Salmon (1900), commenting on the infrequency of ascospores on the living host plants, thought that differentiation might take place only after overwintering on dead tissues. As there is no record of spring infection by ascospores in Britain, undifferentiated cleistocarps from

wheat, barley and oats were exposed to overwintering during the seasons 1947-50. Each year samples were drawn from the weathering material at fortnightly intervals, from October to March, and examined for ascospore formation. The cleistocarps were taken from standing straws, buried straw, dung straw, exposed straw on unthrashed stacks and from wintering stubbles. In 1949 samples were collected from crops grown over a wide range of districts, at dates extending from June to September, and these were also overwintered outdoors. In the 1947-8 samples differentiation was recorded in one cleistocarp only, in November; in 1948-9 and 1950-1 no ascospores nor empty cleistocarps were found in any group under test. In the 1949-50 samples the only ascospore formation occurred on the basal sheaths of the August-collected material in the early December tests and no further development was recorded after this date. These findings confirm the sterility of the cleistocarps after overwintering and it is, therefore, improbable that the ascospores of *Erysiphe graminis* make any direct contribution to spring infection in this country.

Ascospore shower

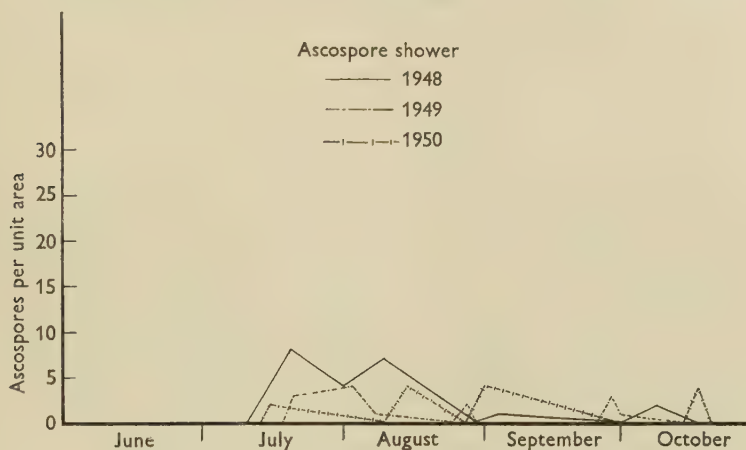
In North America ascospores are shed from September to mid-October and in the U.S.S.R. at the end of July. The extent to which a comparable shower can take place in England has been determined from the spore traps exposed daily at Rothamsted during the seasons 1948-50. Only



Text-fig. 1. Intensity and timing of the conidia showers, 1948-49-50.

conidia within $23-30 \times 8-10 \mu$, the range for the cereal strains, have been recorded but chance inclusions from grass strains, especially on the June traps, have not been overlooked. These are not considered to be significant. The recorded ascospores lie within the range, $20-23 \times 10-13 \mu$, and as cleistocarp development on the grass hosts is relatively infrequent in Britain, inclusions from that source are negligible. In each year peak conidial shed

occurred in June, dropped steeply at the end of July and rose again during October (Text-fig. 1). More significantly, germinating conidia were trapped until mid-July and again in September and October. Ascospores first appeared on the traps in July, the greatest load was carried from mid-July to the end of August and the deposition of ascospores extended to late September (Text-fig. 2). Seasonal variation was expressed in unit load, rather than in time of deposition, but in 1950 a few germinating ascospores were recorded up to late October. These figures, although collected in one area only, establish that ascospores of *Erysiphe graminis* are formed and shed in England during late summer and early autumn. The shower is considerably less heavy than that of the conidia, and coincides with host maturity when conidial abstriction ceases.



Text-fig. 2. Intensity and timing of the ascospore showers, 1948-49-50.

Source of the shower

To determine the source of the ascospore shower, cleistocarps from infected wheat, barley and oats have been examined and recorded over a 5-year period. Collected from plants grown in a wide range of districts, they have been drawn in acropetal succession at dates ensuring examination at all stages of development. The most striking feature revealed by the data obtained is the scanty formation of ascospores in the field and the degree of seasonal variation which occurs. When differentiation does take place they are formed first, and in the greatest number, in the cleistocarps on the basal sheaths and leaf blades and in most seasons ascospore differentiation is restricted entirely to this region. In some years, if early infection is also coupled with early crop maturity, ascospores may also be found on the median leaves but the degree of sporulation in this position is very variable and appears to be linked to seasonal humidity. No case was recorded of ascospore formation on the uppermost leaves or on the glumes.

It can be assumed, therefore, that the stubbles are the principal source of the ascospore shower. Surveys during 1947-51 made at, and subsequent

to, harvest confirm this and also indicate the range of seasonal variation. In 1947 and 1948 very few ascospores or differentiating cleistocarps were found on any stubble examined. In 1949, forty-nine stubbles of wheat and barley and twenty-four of oats were recorded. Ascospores were shed freely on forty-five of the wheat and barley stubbles during August and less abundantly on eleven of the oat stubbles in September. In 1950, seventeen wheat and nine barley stubbles were under observation. Ascospore shed was recorded during August and September on eleven of the wheat and seven of the barley stubbles, and on two of eight oat stubbles examined during the same period. In 1951 both infection and cleistocarp formation was very erratic. No single case of ascospore differentiation or ruptured cleistocarp was found on any of the seventy-five wheat, barley and oat stubbles, from Yorkshire southwards to Devon, which were examined from early August to the end of September.

FACTORS CONTROLLING ASCOSPORE DIFFERENTIATION

Potentiality and shed

Ascospore differentiation can be correlated with certain clearly defined conditions within the asci. Those with contents which are coarsely granular or in any degree withdrawn from the walls never produce ascospores; in most years these fill the greater proportion of the cleistocarps (Pl. 21, fig. 1). Asci with finely granular contents, especially when suspended in a viscous fluid, rich in oil drops, appear to be the potential source of ascospores (Pl. 21, fig. 2). Incomplete or arrested differentiation is not uncommon and all stages may be found within a single cleistocarp (Pl. 21, fig. 3). External appearance gives no indication of potentiality and the smaller, pale-coloured cleistocarps may produce ascospores when the larger, darker forms do not.

The period between full differentiation and dispersal is very brief; the cleistocarps split equatorially and the ascospores are ejected through the aperture, germinating within a few hours of shed (Pl. 21, figs. 4, 5). The factors which control dispersal are not clearly defined, for amongst the shedding cleistocarps some are always to be found which remain intact, although the contained ascospores are viable and may emit germ tubes. The presence, therefore, of ascospores in an unruptured cleistocarp does not necessarily imply subsequent contribution to the ascospore shower.

Host maturity and age of the cleistocarp

Potentiality for sporulation appears to be coincident with a 'ripe-to-spore' condition in the cleistocarp and this to be related to age and host maturity. Differentiating cleistocarps have only been found on senescent host tissues, never on the green leaf, but even amongst these many remain infertile. As ascospore formation is most prolific on the basal sheaths, that is in the oldest cleistocarps, the relationship between age and differentiation has been examined. In 1950-1 winter wheat was sown, six plants per pot, in quadruplicate, at monthly intervals from October to March. Series A was grown in an unheated greenhouse and series B outdoors, and each

sowing was infected with *Erysiphe graminis* at the first leaf stage. In series A, cleistocarps developed on the basal sheaths of the October-sown plants on 1 June, on the November and December sowings on 8 June, and on the January plants on 13 June. In series B, cleistocarps appeared similarly on 8, 13 and 27 June, respectively. In two pots from each sowing in series A, 9 in. polythene cylinders were placed round the base of the plants 10 days after the appearance of the cleistocarps on the basal sheaths so that their further development was under increased humidity. No ascospores were differentiated on any of the uncovered plants in series A, but 64 % of the cleistocarps on the basal sheaths within the polythene covers developed ascospores. Shedding started on 29 July from the October-sown plants and from the remaining sowings on 5 August. In series B differentiation was delayed until 9 September, when it occurred on the basal sheaths only, of all sowings. A vigorous shower followed until 12 September, by which time up to 45 % of the cleistocarps were ruptured and empty, the greatest dispersion occurring on the January-sown plants. No further shed was recorded after this date from the November, December and January sowings, but on the October plants dispersion continued until 29 September. This marked the end of the ascospore shower.

Comparable sowings and inoculations were made with winter barley and winter oats. In the barley series no cleistocarps were developed in the greenhouse, but outdoors they were formed on the basal sheaths and upper leaves of the October-sown plants and on the upper leaves, only, of the later sowings. Ascospores were again differentiated on the basal sheaths of the October sowings and dispersal was completed by 11 September, but no differentiation was recorded in any of the remaining plants. In the oat series cleistocarps were sparsely formed on both basal sheaths and upper leaves on all the sowings, but no ascospore formation was recorded on any plant either under glass or outdoors.

In a further series, C, wheat plants were sown outdoors on 5 November and inoculated with mildew on 23 April, 9 May and 1 June, natural infection appearing on the controls on 16 May. After infection, series C1 was grown under glass and series C2 continued growth outdoors. In C1, cleistocarps appeared on the basal sheaths of the April infections on 8 June, on the May inoculations and the controls on 11 June and on the June infections on 11 July, but in no single case were ascospores differentiated. In C2, the April-inoculated plants bore cleistocarps on 14 June, the controls on 20 June and the May and June infections on 27 June and 27 July, respectively. Ascospores were differentiated on the basal sheaths only of the April and May inoculations and the controls; they were shed from the controls on 9 September and from the April and May inoculations 2 days later.

In 1951-2 wheat, barley and oats, sown in pots under unheated glass and outdoors at fortnightly intervals from September to March, were infected at the first-leaf stage. In the wheat series there was a difference of 23 days between the first appearance of cleistocarps on the basal sheaths of the September and the March sowings but no ascospore differentiation was found on any plant in the series, either under glass or outdoors. These results were paralleled in oats, and cleistocarps were not formed in the barley series.

As the first-formed cleistocarps on the basal sheaths are the most consistent source of ascospores, in those years when differentiation does occur, age would appear to be contributory to sporulation. That it is not the controlling factor is evident in the seasonal variations which occur and from the reaction of these basal cleistocarps to moisture, either under normal precipitation outdoors or with increased humidity under glass. If moisture is also significant in ascospore differentiation, then the oldest cleistocarps located in the humid microclimate at the base of the plant, in closely sown field populations, should be the most favourably placed.

Moisture relations

Wolff (1875) found that undifferentiated cleistocarps of *Erysiphe graminis* produced ascospores when immersed in water for 5-6 days. Salmon (1903) further showed that complete submersion was not essential, provided that moisture contact was maintained. Graf-Marín (1934), working on barley, found that ascospores were most readily differentiated after submergence for 12 hr. at 9° C., followed by incubation at 22° C. Examination of cleistocarps from the three cereals, made during the 6 years 1948-53, confirm that sporulation is closely linked with available moisture, provided that the asci are potential spore formers, i.e. that the 'ripe-to-spore' stage has been reached. No evidence has been found of any consistent increase in ascospore formation, following pre-chilled water treatment and incubation, over controls maintained at constant temperatures or subjected to day-and-night fluctuations. Low temperature is not considered to be a pre-requisite for differentiation in Britain as Graf-Marín found in the U.S.A. In the laboratory, as in the field, the most consistent response to moisture is always found in the cleistocarps from the basal sheaths but in some years, especially on material from the drier, early districts, sporulation may occur in the cleistocarps on the median leaves under high moisture treatment. More generally it is sufficiently erratic to support the view that most of the cleistocarps on these leaves are sterile. In this country the cleistocarps borne on the upper leaves and on the ears rarely produce ascospores under high moisture treatment, but the author has obtained them under these conditions from infected wheat glumes collected in Holland.

Reaction to moisture may be accelerated, in some seasons, by storage. Cleistocarps from the basal sheaths of wheat and barley, which did not differentiate under high moisture when collected in October 1949, did so freely in November after storage at room temperature. No increase in differentiation was obtained after this date, however, either under extended moisture or at a varying temperature range. In 1950, undifferentiated cleistocarps from wheat were collected weekly from 21 June to 9 August, stored at room temperature and subjected to moisture treatment, also at weekly intervals, until the end of November. Ascospore formation was negligible in the June and early July material in all the tests but the cleistocarps collected on 17 July first produced ascospores on 10 August, after 21 days storage, and those gathered on 9 August first produced ascospores on 20 August, after 7 days storage. After these dates a steady rise was recorded in the number of cleistocarps sporulating under the weekly

moisture tests until the end of October, beyond which further differentiation ceased. In 1948, 1951 and 1952, however, when similar collections were made and tested on comparable dates, no ascospores were differentiated in any of the samples under high moisture following storage. Drying of the cleistocarps may, therefore, be significant in some years in so far as it furthers 'ripeness-to-spore' but, as the completion of this stage is closely linked with maturity and environment, the climatic conditions prevailing in any given year influence greatly the reaction to storage.

The cleistocarpic mat is an important factor in ascospore formation, particularly when the mycelium is dense and floccose. The sterile hyphae and the cleistocarp walls are highly permeable but water intake, in amounts sufficient to initiate sporulation, is retarded by air held between the mycelial strands. Even after submergence these barriers may still remain intact and they are a limiting factor for differentiation in the more deeply embedded cleistocarps (Pl. 21, fig. 6). The scanty and erratic development of ascospores on exposed leaves in the field, in seasons when cleistocarps from the same material differentiate freely under controlled moisture in the laboratory, is largely due to the efficiency of the air-locks in the mycelial mat. This is very evident when heavy dews are followed by bright sunshine and drying winds. Under optimum moisture, ascospore differentiation starts within 36 and 48 hr. Short intervals between wetting and drying, i.e. less than 36 hr., do not inhibit differentiation, but once it is initiated it is sensitive to any decrease in moisture. Should drying-out then occur, even for short periods, ascospore formation may not be completed.

Climatic factors

Although the duration of the ascospore shower in corn mildew is fairly uniform from year to year, the intensity varies. This can be related to the climatic conditions prevailing during the growing season and the harvest period. In warm, dry years, when infection and host maturation occur early in the season, most of the cleistocarps on the basal sheaths and a proportion of those on the median leaves reach the 'ripe-to-spore' stage. Subsequent differentiation is then controlled by the moisture available at and following harvest. Long, wet periods initiate vigorous sporulation and a heavy ascospore shower. Intermittent rain, alternating with periods of high temperature and drying winds, depress differentiation on the exposed median leaves and the shower is then restricted to those regions where ground cover maintains a more constant humidity, that is the basal sheaths. A dry harvest with high sunshine and low humidity inhibits ascospore formation in the field and the shower is correspondingly scanty. In a late, wet spring, when both infection and host maturity are delayed, few cleistocarps ever reach the 'ripe-to-spore' stage and those which do are located on the basal sheaths. If the harvest in such a year is wet, the very light ascospore shower will be derived solely from the basal cleistocarps, but if conditions are very dry it may be negligible.

The stubbles are thus the main source for the ascospore shower, even in those years when the median leaves carry a proportion of fertile cleistocarps. Where, in a dry, early harvest, sporulation on the stubbles has been

restricted through lack of moisture, a secondary shower may occur late in September, following the autumn dews. Beyond this date the cleistocarps which remain intact on the stubbles are usually found to be abortive.

VOLUNTEER INFECTION

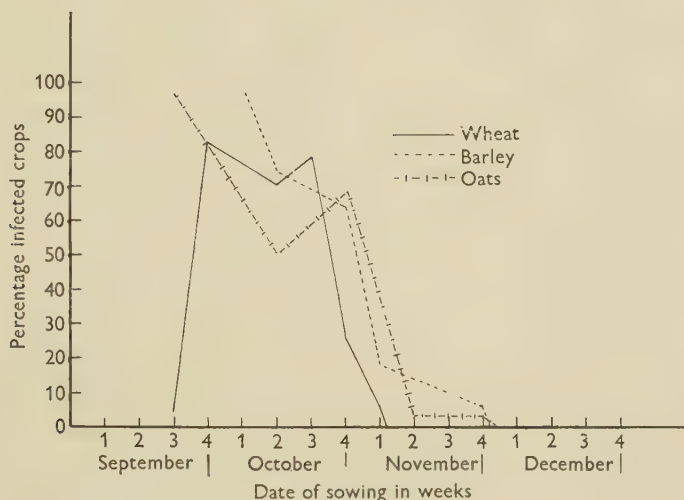
In wheat and barley, the ascospores are the main source of autumn outbreaks of cereal mildew. Direct infection may occur on early sowings but the greater part is indirect, derived from mildewed stubble volunteers, previously infected by ascospores. The extent to which autumn infection occurs in these two cereals is, therefore, related to the intensity and duration of the ascospore shower and to the growth of the volunteer plants. High moisture during the post-harvest period, which favours a vigorous shower, also favours rapid volunteer growth, and in such years infection on both volunteers and autumn crops is heavy. When drought conditions prevail during August and September, the ascospore shower is delayed and there is little early volunteer growth. When germination does take place in October on the unploughed stubbles, most of the ascospores will have been shed by the time the seedlings reach the first-leaf stage, and such infection as may occur will be of little significance. In late, wet seasons with low fertility in the cleistocarps, the ascospore shower is too scanty to produce serious infection on the volunteers and this is still further reduced if induced grain dormancy, due to high moisture content, delays germination beyond the critical infection period.

In oats the position is somewhat different. Many modern oat varieties produce infected late tillers and this semi-perennial habit is often accompanied by reduced cleistocarp formation. Gorlenko (1942) relates this drop in cleistocarp number, when it occurs in the grasses, to perennial habit and prolongation of the conidial stage. Most of the tillers continue growth after harvest and carry sporulating lesions which are the source of infection for the heavy attacks of mildew on early-sown winter oats and for the volunteers. Some ascospore differentiation does also occur but it is considerably less than that found in wheat and barley, and it is probable that the ascospores make only a minor contribution to winter infection.

AUTUMN CROP INFECTION

To examine the extent to which mildew occurs in autumn-sown cereals, surveys were made of 439 crops of winter wheat, barley and oats during the autumn-winter periods of 1948-51. In 1948-9, 34 crops of wheat, out of 178 examined, carried mildew. The maximum number of infected crops was recorded in the late September and October sowings, there was a marked drop in early November and no infections were found in crops sown later than this (Text-fig. 3). In 1949-50, 62 autumn wheat crops were examined, 17 of which were infected, and the same progressive decrease in infection was found as the date of sowing was delayed. In the barley crops examined in 1948-9, none was sown before the second week in October. A drop in the number of infected crops was recorded as the sowing date was delayed but infection on a decreasing scale was continued throughout November (Text-fig. 3). This was paralleled in 1949-50.

Winter oats showed a decrease in the number of infected crops similar to that found in wheat but, after the marked drop at the beginning of November, cases of infection were recorded in crops sown up to the end of that month, as in barley. Infection in all autumn cereals in 1950-1 was too low for comparable counts, but in those few cases where mildew was recorded the same correlation between infection and date of sowing was observed.



Text-fig. 3. Incidence of infection in autumn-sown cereals.

Incidence of disease within the infected crops also decreased as the date of sowing was delayed. In wheat the figures recorded dropped from 82 % in crops sown before the end of the second week in October, to 6 % in the fourth week and to less than 3 % in the November sowings. In both autumn-sown barley and oats the incidence ranges followed the same trend.

WINTER BUILD-UP OF INFECTION

Resistance of the later-sown cereals to mildew and removal of the sources of infection are the most significant factors in the drop in disease which follows delayed autumn sowing. Contrary to Gorlenko's (1940) findings in Russia, the mycelial mat in this country does not become brown and dormant during the winter, abstricating conidia only in the spring. Mycelial growth and sporulation are continuous throughout the winter, except during periods of severe frost, and the conidia germinate over a wide range of temperature (Pl. 21, fig. 7). Germ tubes can be emitted at 0.5° C., although further elongation does not usually occur below 3° C. and cases of *in situ* germination have been found in February. Conidia produced in the field during the winter readily infect healthy plants under greenhouse conditions. Despite this parasite vigour, however, there is very little build-up of disease in the autumn-sown cereals during the winter months, after the initial infection, although examinations made of overwintering

aphids show that considerable numbers of conidia are transferred on the bodies of these insects from the sporangial lesions to healthy leaves. In crops sown from November onwards, such infections as do occur are usually confined to the first leaf, often to single lesions, and it is not easy to infect the second leaf with mildew between December and February, especially in wheat. Disease establishment is also difficult in those springs, following wet winters and heavy soil leaching, when cereal crops show the typical yellowing associated with nitrogen deficiency. Tapke (1951) has shown that a temporary resistance to mildew infection or 'toughening' can be induced by low temperatures and reduced nitrogen. Last (1953, 1954) has demonstrated that, in wheat, susceptibility to mildew infection is directly related to rising temperature and to increase in nitrogen status with its consequent effect on growth rate. He also finds that nitrogen deficiency in the host plant is associated with resistance. Thus the drop in infection, following upon delayed autumn sowing, would appear to be related to falling temperatures, reduction in available nitrogen and retarded growth rate in the host, all of which obtain during late autumn and winter.

The clover ley stubbles, where spring cereals are used as nurse crops, present a different problem. Surveys of these during 1949-51 show that the humidity among the young clover plants after harvest favours a heavy ascospore shower and a high proportion of germinating volunteers, whilst the high nitrogen status of the soil encourages growth and disease establishment. In the case of oat nurse crops the infected late tillers make a further contribution. Spring cereal volunteers can survive the winter under the protection of the ley and, contrary to the findings for the late-sown autumn cereals, there is a continuous build-up of actively sporangial lesions on these plants throughout the winter. The extent to which this can occur is shown by twenty-four oat volunteers, twenty-two of which were mildew-infected, which were recorded on 14 February 1950, following 8 days heavy frost. The plants carried 123 tillers with 378 expanded leaves, 117 of which were diseased. Of the 799 lesions examined, 644 were sporangial freely. Comparable figures were recorded for barley nurse crop volunteers, and although spring wheat is less frequently used on the leys, there are indications that similar conditions exist. Ley stubbles, therefore, not only provide an effective source of autumn infection but can be important centres for early infection in spring.

An outstanding feature of the very large numbers of overwintering plants examined during the course of this investigation has been the hard grazing by rodents and vermin during periods of severe weather. *Erysiphe graminis* lesions have never been found on plants where part or all of the first or second leaves have been removed in this way. Low spring infection, following upon a hard winter, has been reported by many workers, and this biotic factor may be significant in the reduction of foci for spring infection.

DISCUSSION

The ascospores are a significant link in the spread of cereal mildew in this country and continuity of infection in successive crops of wheat and barley is dependent upon the intensity and timing of the ascospore shower, the

stand of volunteer plants and the date of the autumn sowings. The 'ripe-to-spore' stage in the ascus, which may be controlled by the environment under which the cleistocarps develop and the degree of senescence in the host tissues on which they are borne, is followed by ascospore formation only if there is a sufficiency of moisture to initiate differentiation. In most seasons the optimum conditions for sporulation are located at the base of the plants, so that the stubbles are the main source of the ascospore shower. Maximum shed takes place under high humidity at or following harvest, conditions which are equally favourable for the rapid germination and growth of stubble volunteer seedlings and their infection by the ascospores. Conidia from these diseased plants infect adjacent autumn-sown crops, but direct ascospore infection can occur in very early sowings and to some extent when drought conditions delay the shower. Date of sowing bears a direct relationship to autumn infection and there is a marked drop in mildew incidence in wheat and barley crops sown after mid-November. There is, however, little spread of infection from plant to plant amongst these crops during the winter months, even under mild weather conditions, although active growth and sporulation continues in the existing lesions throughout this period. This is significant in so far as the autumn infection determines the incidence of foci from which the spring outbreaks arise.

In oats, infection of the autumn-sown crops is less dependent upon the ascospores. The late, actively growing tillers, produced by many spring varieties, become infected towards the end of the conidial shower and the disease is transferred from these either directly to the winter-sown oats or through volunteer seedlings. As in wheat and barley, susceptibility to mildew and subsequent spread in oats is also related to the date of autumn sowing, and infection may be very heavy in the early-sown winter varieties.

The incidence of mildew amongst cereal volunteers on young clover leys does not remain static during the winter months. Under the protection and high nitrogen status of the ley there can be a steady increase in the number of lesions throughout this period and a corresponding rise in number of infection foci. This makes the clover leys important, and often overlooked, centres for mildew outbreak in the spring. As ascospore differentiation and spread are so closely linked with the conditions prevailing during the growth of the crop, the probable intensity and timing of the ascospore shower can, in some degree, be predicted in any given year. Similarly, the extent to which mildew may develop in the early spring can be forecast from the climatic conditions of the previous early autumn and the amount of infection established in the overwintering cereals at that time.

Any operation, such as stubble cleaning and ploughing immediately after harvest or delayed sowing of winter cereals, whereby the sources of the ascospore shower and the volunteers are removed or the period between harvest and autumn sowing extended, lessens the chance of epidemic outbreak in the following spring. The autumn grazing of clover leys, especially where there is a heavy growth of infected volunteers, and the spring grazing of early-sown autumn cereals, also reduces the number of foci from which corn mildew can build up in the following season.

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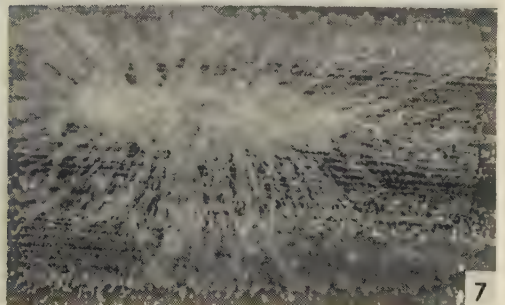
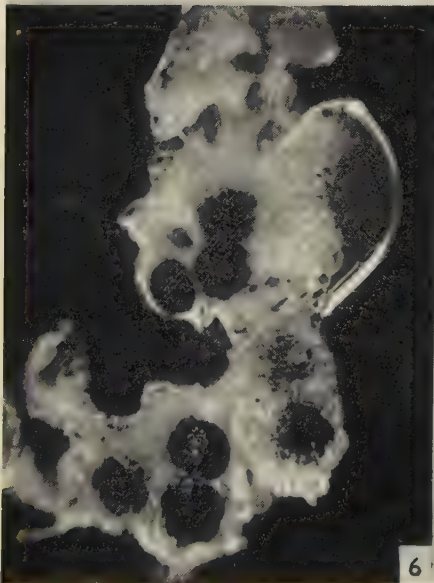
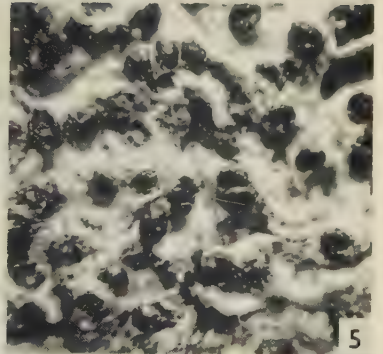
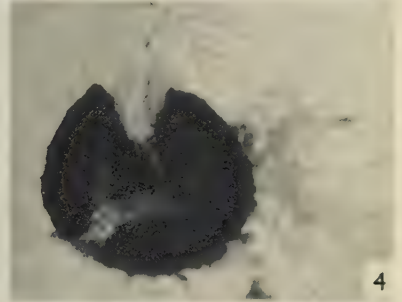
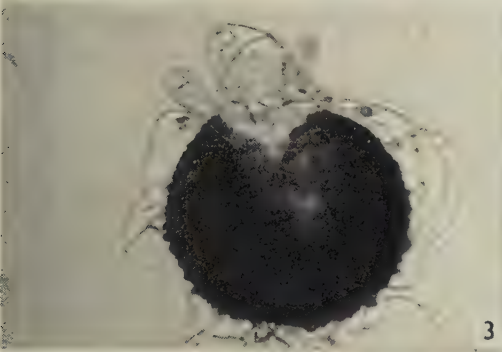
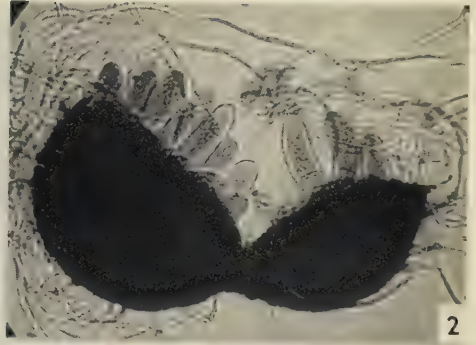
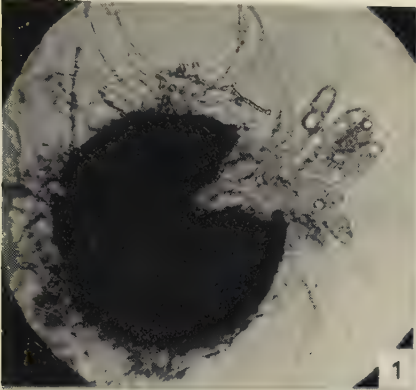
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EXPLANATION OF PLATE 21

Erysiphe graminis

- Fig. 1. Abortive asci with coarsely granular contents. $\times 150$.
- Fig. 2. Potential ascospore-bearing asci with uniform contents. $\times 150$.
- Fig. 3. Arrested ascospore differentiation within the cleistocarp. $\times 150$.
- Fig. 4. Fully differentiated ascospores. $\times 135$.
- Fig. 5. Ruptured cleistocarps on the basal sheaths. $\times 20$.
- Fig. 6. Air-locks within the cleistocarpic mat. $\times 35$.
- Fig. 7. Growth and sporulation in overwintering lesions on wheat. $\times 30$.

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REVIEWS

Dawn in Andromeda. By E. C. LARGE. (London: Jonathan Cape.) 282 pp.
Price: 15s.

Mr Large is not a prolific writer. We have waited 16 years for a successor to *Sugar in the Air*, *Asleep in the Afternoon* and *The Advance of the Fungi*. Now we are rewarded by *Dawn in Andromeda*. This is an allegory recalling *The Pilgrim's Progress* in its freshness and sense of purpose. Man, it seems, has not proved an unqualified success on Earth, so God and Mother Nature arrange for ten selected men and women to be transported to an unpeopled planet in Andromeda—a planet whose evolution has been similar to our own, up to a point. Here, emerging naked from the sea one misty morning, they set about their task. That morning the first fungus foray is held in Andromeda, and the reader will feel a sense of relief when one of the party recognizes *Laccaria laccata* var. *amethystina*. Such is the setting of the story, tranquil at first but developing a climax of excitement as the years go by.

Like many great books, *Dawn in Andromeda* is complex; it holds the reader's interest on a number of different planes simultaneously. The story itself fascinates with its delineation of the main performers, their reactions to the needs of their community, the palimpsest of Earth which each one carries, and the characters of their children. Within the story is embedded a history of the origin and development of technology; a vivid recital of the major discoveries of prehistoric men and women, such as kindling fire, twisting thread, brewing, smelting, breeding and selecting crop plants. The development of the technologies into scientific disciplines is telescoped into a single generation. Deeper down, the allegorical theme running through the whole work gives a diagnosis for our age, and traces out an anti-clerical but deeply religious philosophy.

Dawn in Andromeda is full of the pranks of a lively mind—the felicitous naming of the characters for example—and its style is a conversational triumph.

P. H. GREGORY

Biochemistry of Autotrophic Bacteria. By HOWARD LEES. (London: Butterworths Scientific Publications. 1955.) Pp. viii + 112, 6 text-figures.
Price 21s.

Although this attractive and lucid little book is designed for university students taking an ordinary or honours degree in biochemistry it could be read with interest by many mycologists. The approach is frankly biochemical and after an introductory discussion of the autotrophic way of life, chapters are devoted to the sulphur, the hydrogen, the nitrifying, other chemosynthetic, and the photosynthetic bacteria. The less biochemically minded reader will probably regret the exclusion of any consideration of the morphology and general bacteriology of the forms discussed (an omission which surely unbalances the book, even for biochemists) but he should be interested in the example of speculation on possible evolutionary relationships from biochemical evidence and welcome the clues given in the final chapter to the ecological importance of autotrophic bacteria.

G. C. AINSWORTH

Bibliographie der Pflanzenschutzliteratur, 1951. By J. BÄRNER (editor). (Berlin-Dahlem Biologische Bundesanstalt für Land- und Forstwirtschaft, 1955.) xlv + 420 pp. Price: not stated.

This volume cites 12,500 titles of papers published during 1951. Other volumes are promised shortly to close the gap, 1946–1950, in the great Bibliography started by Morstalt to cover the world's literature on plant protection from 1914 onwards.

'The predacious fungi: Zoopagales and Moniliales.' By C. L. DUDDINGTON.
Biol. Rev. **31**, 152–193, 1956.

This review, with its more than 200 references, is a valuable complement to the author's paper on technique for handling predacious fungi which appeared recently in these *Transactions* (**38**, 97–103, 1955).

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ERRATA

Volume 38, part 4

- P. 445, line 8. *For* 'cospores' *read* 'oospores'.
 P. 448. Delete last line.